ACTA RADIOLOGICA

FOUNDED IN 1921 BY GÖSTA FORSSELL

PUBLISHED BY THE SOCIETIES OF MEDICAL RADIOLOGY IN DENMARK FINLAND NORWAY AND SWEDEN

ONCOLOGY

RADIATION THERAPY, PHYSICS AND BIOLOGY

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RADIATION THERAPY PHYSICS AND BIOLOGY

Vol 19 1980 Fasc 2

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ACTA RADIOLOGICA

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Fasc 1

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1980

EDITORIAL

With this issue Acta Radiologica begins publication in a new form after having had the same appearance and been produced by the same processes ever since it was founded in 1921 by Gosta Forssell The design of the cover for the individual numbers has however been changed the table of contents being moved in 1951 to the front page in order to make it easier for readers to obtain information on the articles in the issue without having to open it And in 1963 the journal was divided into two series one on diagnosis and one on radiation therapy physics and biology

It is of great importance that the illustrations in a periodical presenting radiologic material should be of the highest quality If a reader is able by examining the illustrations to satisfy himself that the author has interpreted the images correctly he will also find it easier to evaluate the conclusions drawn in other words form a better opinion regarding the value of the article Even with blocks of high quality it has sometimes been necessary because of the size of the journal to present illustrations which in some cases have been too small with the result that some of the details could not be readily distinguished With increasing refinement of the imaging techniques and as ever smaller details have proved to have diagnostic significance a sense of dissatisfaction with the format of the journal has gradually developed A change over to a larger size has in fact been under consideration for several years but has been deferred until a change to a more modern method of production could be effectuated at the same time

As from the present issue the journal will be produced by means of modern computer generated phototypesetting and offset printing Other periodicals have earlier adopted the offset technique but in the opinion of the editors of this journal images produced with this process have only recently attained a quality that can be considered acceptable for use in radiologic publications Thus the change to offset was not made until comparative trials had brought conviction that the new method for image reproduction has reached a stage giving results comparable to those obtained with the old block making system

Apart from the change in appearance and the new method of production the principles of the journal will remain unchanged This means that the two series will continue to be published also in the future The diagnosis series will as before be a journal of general radiology with sidelines on some special fields such as neuroradiology and pediatric radiology In the future as in the past considerable attention will be paid to the layout in order to ensure that the articles are presented in a clear and esthetically attractive form

One of the reasons why Acta Radiologica was instituted was the desire to have a forum where experiences and results of research in radiology and allied fields in the Nordic countries could be published so that readily available information could be obtained regarding developments trends and future prospects in these countries As the speech communities covered by these countries are small the articles had to be published in one of the major languages of the world if they were to be available to readers in other areas In the beginning papers in English German and French were accepted However English has gradually become the dominant international language in the medical world holding a position almost comparable to that of Latin in the world of science during the Middle Ages and the centuries immediately following that period and the journal therefore subsequently adopted the policy of publishing articles only in English Summaries in German and French were retained but from this issue onwards they will be abandoned since it may be considered hardly possible these days to follow developments in any branch of medicine without a

knowledge of English Acta Radiologica will therefore from now on be published exclusively in English

Although the journal was originally intended to be a forum mainly for the Nordic nations its pages have always been open to authors from other countries as well Many important articles by non Nordic writers have also been gratefully accepted for publication It is hoped that these contributions will not only continue but also increase in number Problems will thereby receive more all round treatment and comparisons with other points of view facilitated which should be to the mutual advantage of both Acta Radiologica and its readers

In recent years new diagnostic methods based on imaging techniques have been introduced ultrasound, scintigraphy computer tomography and possibly still others such as magnetic resonance will appear in the future Opinions differ it is true regarding the appropriate speciality to which these methods should belong Acta Radiologica holds most emphatically that all imaging techniques should be concentrated to the radiologic departments where the basic skills are centered Furthermore the methods are as a rule such that the solution of a diagnostic problem is seldom gained by one method alone the methods are supplementary to each other as well as to conventional radiology Thus the most suitable way to achieve this is t assemble them all at one institution In accordan with this opinion Acta Radiologica has publishe and will continue to publish articles on all imaging procedures In order to emphasize more clearly than previously the fact that the diagnosis section the re series is a journal of medical imaging information to this effect has been added to the first page of the cover It further appears that the journal will also accept reports on physiologic sequences analysabl by radiologic methods in the widest sense

Like other clinical specialities oncology needs the methods offered by diagnostic radiology b otherwise they have little in common They should accordingly be practised by different specialists As a consequence of this Acta Radiologica as the onl radiologic periodical has run and will continue t run two series one diagnosis and one oncology t latter comprising not only radiation therapy but al chemotherapy and radiation physics and biology

With this declaration of its programme Ac Radiologica is presented to the readers in its ne form

FROM THE DEPARTMENTS OF RADIATION PHYSICS AND ONCOLOGY AND RADIATION THERAPY UNIVERSITY OF AARHUS DK 8000 AARHUS DENMARK

LARYNGEAL CARCINOMA

IV Analysis of treatment results using the Cohen model

M HJELM HANSEN

This report presents an analysis of treatment results in which the biologic effect of irradiation is estimated by using the Cell Population Kinetic Model proposed by COHEN (1971) The reader is encouraged to review all cited papers by COHEN as well as the one by SACHER & TRUCCO (1966) in order to gain an understanding of the details of this model

Material, Methods and Definitions

The material consisted of 308 patients (94 supraglottic 211 glottic 3 subglottic) treated by primary irradiation for laryngeal carcinoma in the period August 1963 to August 1972 Clinical details regarding the total material of 359 patients from which the analysis group has been selected are given in Part I (JØRGENSEN et coll 1979) Details regarding the analysis group of 308 patients as well as an analysis of treatment results using the Ellis model are given in Part II (HJELM HANSEN et coll 1979) and the treatment results in relation to a microscopic score are found in Part III (LUND et coll 1979) The clinical data on which the analysis is based refer to December 1976

Statistical method The chi square test was used to test for correlations when frequencies are given as minimum or estimated values If frequencies are calculated by use of the life table method (Fig 4) the logrank test was used (PETO et coll 1977)

Minimum absorbed dose (D_{min}) to the tumour was taken to be 95 per cent of the calculated central absorbed dose (D_c) For larger tumours this was a claim for inclusion in the analysed group and for smaller tumours (well within the fields) the build up and build down of dose at the intersection of air and tissue (trachea and laryngeal cavity) will cause the minimum absorbed dose to be approximately 5 per cent lower than the central absorbed dose obtained in ordinary dose planning (KOSKINEN & SPRING 1973) Minimum absorbed dose is used in analysis of local recurrence

Maximum absorbed dose (D_{max}) is defined as the absorbed dose at the central axis in a depth of 0 5 cm Maximum absorbed dose was used in the analysis of complications Usually with the technique used today very little difference occurs between D_c and D_{max} However in the early series some patients received treatment with alternating fields and while D_c is associated with a number of equal fractions D_{max} is associated with alternating large and small fractions which may produce a different biologic effect than a number of equal fractions

The Cohen model is based on calculation (separately for each individual tissue in question) of survival fraction obtained after each individual fraction of irradiation and of repopulation during the time intervals between the individual fractions The survival curve is assumed to be given by the equation

$$S=e^{-jD}[1-(1-e^{-kD})^N] \qquad (1)$$

where S is the surviving fraction D is the absorbed dose (tumours D_{min} normal tissues D_{max}) and j k

Submitted for publication 21 March 1979

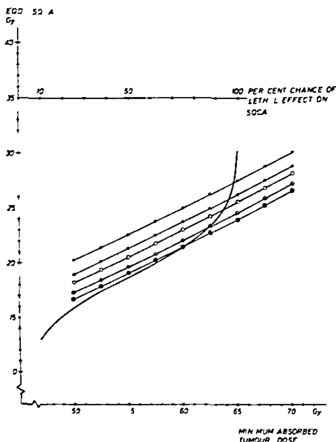


Fig 1 Graph for determination of EQD1 SQCA and prognosis according to the Cohen model used on a schedule of 30 fractions delivered in 41 days Tumour size ○ 1 cm ● 2 cm ○ 3 cm ⊖ 5 cm ● 7 cm

and N are constants which must be specified for the tissue in question

After termination (T=0) of a treatment resulting in a surviving fraction of S(0) repopulation is thought to take place after the logistic formula of SACHER & TRUCCO

$$S(T)=\frac{H\ S(0)}{S(0)+(H-S(0))e^{-LHT}} \qquad (2)$$

S(T) is the surviving fraction at time T H is the possible maximum value that S can reach (depends on previous treatment) and L is a growth constant to be specified for each individual tissue (In some recent publications on the model COHEN is omitting the factor H in the exponential expression which however may change the value of L)

Although eq 2 is derived for normal tissues only (in which case H has a maximum value of 1) it is used for tumour tissues as well by allowing H to exceed the value of 1 i e tumour growth beyond the initial size is possible

Procedure of calculation

(1) Previous treatment has resulted in a surviv fraction of size S(0) (This means S(0)=1 if no pri ous treatment has been given)

(2) During the time from termination of previ treatment to next fraction repopulation will t place and cause the surviving fraction to incre according to eq 2 In order to use this equation must be known

For tumour tissues H is a constant in the rang to 2 while for normal tissues H depends on previo treatment and is calculated from the equation

$$H=e^{G}\prod_{i=1}^{i=n} S_i \qquad H\leq 1$$

where n fractions with the individual effects S= i=1 2 3 n (eq 1 with $D=D_i$ i=1 2 3 constitute the previous treatment and G is a co stant to be specified for the tissue in question If t value of H calculated from eq 3 is greater than then H=1

(3) The surviving fraction immediately befo next fraction is then obtained (S1)

(4) The separate effect of next fraction (S2) calculated from eq 1

(5) The total surviving fraction immediately aft this last fraction is then S(0)=S1×S2

(6) Sequence 1 to 5 is repeated as long as furth fractions are given

(7) After termination of the treatment a final val (S_F) of surviving fraction is obtained also given

$$S_F=\prod_{-0}^{-1}\frac{H(i)\,S_{i+1}}{S(i)+[H(i)-S(i)]e^{-H(i)LV(i)}} \qquad ($$

where H(i) is the value of H after fraction No i (e 3) S(i) is the total surviving fraction immediate after fraction No i while V(i) is the interval b tween fraction No i and i+1 (H(0)=S(0)=1) S_i i the separate effect of fraction No i+1 (eq 1)

Correction for tumour size is performed by mult plying S_F by the factor Z^Y where Z is the tumou size in cm and Y is a constant to be specified fo each individual type of tumour This correction merely a mathematical convenience utilizing th fact that if a certain survival fraction is obtained larger number of tumour cells will be left in a larg tumour than in a smaller one The prognosis of th larger tumour is assumed to be similar to the pro gnosis of the smaller tumour treated less intensivel

Table 1

Values of the constants used in the present analysis

	G	j rad⁻¹	k rad⁻¹	L day⁻¹	N	Y	μ_a	σ_a
SKIN	8 72	0 00415	0 00486	0 34	13 0	0.57	4 70	1 93
VASC	18 00	0 00790	0 00370	0.23	6 6	0.33	4 00	2 00
GUT	18 00	0 00760	0 00470	0 13	21 1	–	4 70	2 20
SQCA	1.50	0 00480	0 00620	0 07	11 0	2 00	7 87	2 01

The reason for including the parameter GUT (significant long term sequelae in the gastrointestinal tract) although this has no relevance for laryngeal carcinoma, is that this parameter appears in the discussion of the split-course technique

Table 2

Values of the parameters EQD1 SQCA and EQD1 VASC in a treatment schedule of 30 fractions in 41 days compared with the values of the parameter PT_{1800} (Ellis model)

Central absorbed dose (Gy) D	Minimum absorbed dose (Gy) D_{min}	EQD1 SQCA (based on D_{min})		EQD1 VASC (based on D)		PT_{1800} (based on D) ret
		Tumour size (cm)	Gy	Field size (cm)	Gy	
57 00	54 15	1	22 [illegible]	6	12 3	1 612
		3	[illegible]	8	14 4	
		5	19 3	10	16 2	
60 00	57 00	1	23 6	6	13 4	1 746
		3	21 5	8	15 6	
		5	20 6	10	17 7	
63 00	59 85	1	[illegible]5 0	6	14 4	1 883
		3	23 0	8	16 9	
		5	22 1	10	19 2	
66 00	62 70	1	[illegible]6 4	6	15 6	2 025
		3	24 4	8	18.2	
		5	23 5	10	21.2	

(i e to a higher survival fraction) in which case a similar number of tumour cells would be left in the two tumours

In the present analysis of local recurrence (Figs 2 3) the tumour size is taken to be the largest dimension of the tumour (an integer number of cm) which means that a tumour of dimensions 3 cm×1 cm is corrected as a tumour of size 3 cm×3 cm Since such two tumours obviously contain different numbers of cells some problems may arise

The difference in the value of EQD1 SQCA for a 5 cm×5 cm tumour and a 5 cm×3 cm tumour is about 0 5 Gy However if the width of the tumour is much smaller than the length (as typical in glottic stage I) the difference in the value of EQD1 SQCA may be 1 0 to 2 0 Gy At the steepest part of the theoretical response curve (Fig 1) this may be associated with a difference in local recurrence of 10 to 20 per cent which indicates that correct assessment of tumour size is an important point especially with respect to smaller tumours

The analysis presented in Fig 4 contains the assumption that tumours in glottic stage I of size (length) 1 cm 2 cm and 3 cm in fact represent tumour areas of 1×0 3 cm² 2×0 4 cm² and 3×0.5 cm² The size of these tumours is then taken to be the diameter of circles with areas 0 3 cm² 0 8 cm² and 1 5 cm² respectively

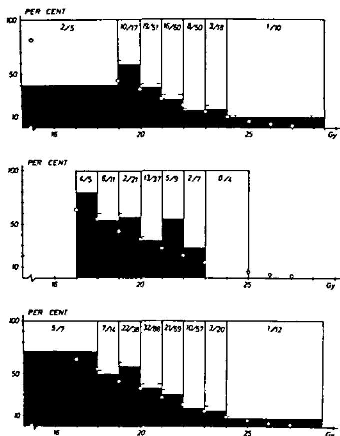


Fig 2 Observed frequencies of local recurrence as function of EQD1 SQCA (Gy) compared with the Cohen model (white circles) Black columns indicate minimum frequencies while stippled lines indicate estimated frequencies Upper Glottic carcinoma (211 patients) Middle Supraglottic carcinoma (94 patients) Lower Glottic and supraglottic carcinoma (305 patients) Correlations (estimated frequencies) in parentheses Glottic—$p<0.005$ $f=4$ ($p<0.001$ $f=4$) Supraglottic—$p<0.01$ $f=1$ ($p<0.025$ $f=2$) Total—$p<0.0005$ $f=4$ ($p<0.0005$ $f=4$)

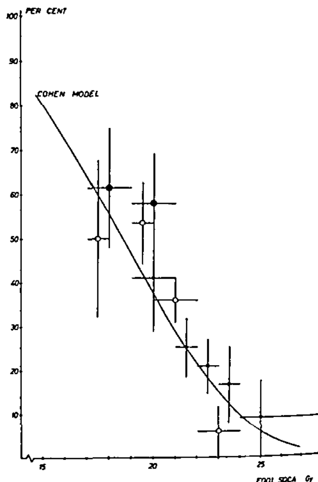


Fig 3 Observed frequencies (minimum values) of local recurrence according to tumour size The standard deviations are calculated from the binomial distribution Correlations 3 to 4 cm $p<0.025$ $f=2$ Size of tumour ● 1 to 2 cm ○ 3 to 4 cm ● 5 to 7 cm

Correction for field size During the computation of S_F a corrected value (U_i) of the absorbed dose (D_i) is used The corrected value is calculated from

$$U_i = \left(\frac{d}{10}\right)^{Y} D_i \qquad (5)$$

(suffix i is used because the individual fractions may be of different size) d is the diameter of the field in cm (i e the side of the equivalent square field) and Y is a constant to be specified for the normal tissue in question This correction is a mathematical convenience similar to the correction for tumour size

(8) Having performed the corrections the equivalent single dose (EQD1) may be calculated as the value of D which inserted into eq 1 yields the final corrected surviving fraction ($S_{F\,corr}$)

$$S_{F\,corr} = e^{-jEQD1}(1-(1-e^{-kEQD1})^{N})$$

and since $kEQD1 \sim 10$ $e^{-kEQD1} \ll 1$ and the binomial expansion $(1-e^{-kEQD1})^{N} \sim 1-Ne^{-kEQD1}$ may be used Thus $S_{F\,corr} \sim Ne^{-(j+k)EQD1}$ or

$$EQD1 = \frac{2.303(\log_{10} N - \log_{10} S_{F\,corr})}{j+k} \qquad (6)$$

(9) The answer from the Cohen model may then be to state the surviving fraction the equivalent single dose or the probability of a specific biologic effect which is expressed as a cumulative normal distribution (function of $Q=-\log_{10} S_{F\,corr}$) by specifying the mean value (μ_Q) and the standard deviation (σ_Q)

COHEN has estimated the values of the constants j k N G L Y μ_Q and σ_Q for a number of tissues among which are normal skin (SKIN) vascular stroma (VASC) and squamous cell carcinoma (SQCA)

The biologic effects in question are SKIN normal

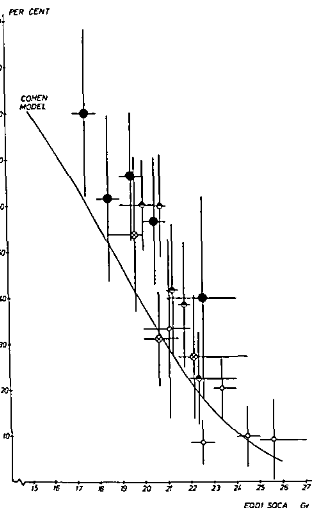


Fig 4 Observed frequencies (10 year life table values) of local recurrence according to tumour location and stage (UICC 1972) The standard deviations are calculated from the Greenwood approximation Larynx (304 patients O glottic stage I ⊖ glottic stage II and III ⊗ supraglottic stage I and II ● supraglottic stage III and IV Correlation glottic stage II and III p <0 05 f=2

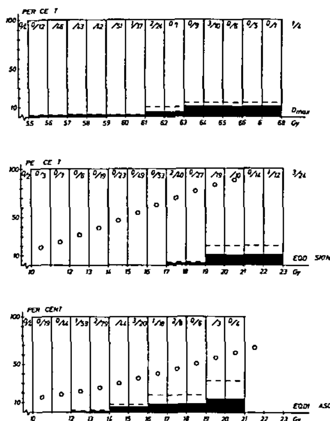


Fig 5 Observed frequencies of late edema as function of D_{max} EQDI SKIN (D_{max}) and EQDI VASC (D_m) Black columns indicate minimum frequencies while stippled lines indicate estimated frequencies The figure includes 3 patients in the subglottic group Circles indicate the risk of dry desquamative reaction (SKIN) and of telangiectasis (VASC) according to the Cohen model

skin tolerance corresponding to a dry desquamative reaction VASC late effect in the vascular stroma such as telangiectasis or endarteritis SQCA long term local control of epidermoid carcinoma The values of the exponents used in the present analysis appear in Table 1

It should be stressed that such values are subject to change since they are derived from statistical analysis of clinical data and may be recalculated when more reliable data are provided All calculations in the present analysis have been performed on a computer using a version of COHEN s RADLOG program (1973) kindly provided by COHEN

Example Some results of calculation using the Cohen model (SQCA) on a fractionation schedule of 30 fractions delivered in 41 days appear in Fig 1

A minimum absorbed dose of 55 Gy delivered to a tumour of size 1 cm results in a value of EQD1 SQCA~22 5 Gy which according to the model is associated with a chance of lethal effect of approximately 82 per cent The same treatment given to a tumour of size 2 cm would result in EQD1 SQCA~21 2 Gy corresponding to approximately a 72 per cent chance of local control while the same treatment given to a tumour of size 7 cm would result in EQD1 SQCA~19 Gy and 53 per cent chance of local control

Table 2 shows values of EQD1 SQCA and EQD1 VASC for some combinations of tumour size and field size in the treatment schedule of 30 frac

Table 3

Comparison between observed and calculated frequencies

Period (most common schedule)	Observed frequency (minimum)		Calculated frequency (per cent) (the working hypothesis)
August 1963 – June 1970	Local recurrence	76/208~36 5±3 3%	33
(57 Gy/30 frac/41 days)	Late edema	5/208~ 2 4±1 1%	6
July 1970 – Aug 1972	Local recurrence	25/97~25 8±4 4%	23
(60 Gy/30 frac/41 days)	Late edema	6/97~ 6 2±2 4%	10

tions delivered in 41 days The values are also compared with the values of the parameter PT_{1800} used in the Ellis model

Results

Local recurrence A total of 103 cases (42 supraglottic 59 glottic and 2 subglottic) of local recurrence were analysed (as of December 1976) The observed frequencies of local recurrence in the glottic the supraglottic and the total group as function of EQD1 SQCA (D_{min}) are given in Fig 2 Black columns indicate minimum frequencies while stippled lines indicate estimated values (glottic stage I corrected for death from intercurrent disease within 6 years supraglottic stage III and IV corrected for death within 2 years and all other groups corrected for death within 4 years)

A very strong correlation exists between local recurrence and EQD1 SQCA This correlation is in fact very close to the one proposed by COHEN although the observed frequencies of local recurrence are usually slightly higher than predicted from the model However this could easily be corrected merely by adjusting the values of μ_Q and σ_Q

Automatically a bias is presented in Fig 2 since larger tumours (i e advanced stages) will appear with lower values of EQD1 SQCA while smaller tumours will appear with larger values of EQD1 SQCA if the actual treatments are equal This is one of the advantages of the Cohen model since it allows simultaneous analysis of the whole group irrespective of tumour location and stage Theoretically any tumour treated to a specific level of EQD1 SQCA should have a prognosis corresponding to this level (Fig 1)

Observed frequencies (minimum values) of local recurrence according to tumour size (largest dimension of the tumour) appear in Fig 3 which sh[o] that observed frequencies—at least for tumour[s] size less than 5 cm—follow the model Howe[ver] since minimum frequencies are approximately o[f] 90 per cent of the estimated frequencies it is evid[ent] that observed values are slightly higher than p[re]dicted from the model

Observed frequencies (10 year life table valu[es]) according to tumour location and stage are given [in] Fig 4 As discussed under correction for tum[our] size an assumption about the size of glo[ttic] tumours stage I is made in this analysis

When the standard deviations are considered [it] must be admitted that it is possible that equal va[lues] of EQD1 SQCA does in fact mean equal risk of lo[cal] recurrence irrespective of tumour location a[nd] stage It is also evident that the true response cu[rve] most likely is steeper than indicated by the Coh[en] model The correction as to size in glottic tumou[rs] stage I changes the distribution of the total in a w[ay] that disturbs the frequency figures in Fig 2 Ho[w]ever the correlation is still valid

Significant correlations between local recurren[ce] and EQD1 SQCA are found for tumours of size 3 4 cm (p<0 025 f=2) and for the group glot[tic] tumours stage II and III (p<0 05 f=2) as was t[he] case in the analysis using the *Ellis model* (Part [] HJELM HANSEN et coll)

Late edema A total of 11 late edemas were o[b]served (9 in the supraglottic group and 2 in t[he] glottic group) Fig 5 gives the observed frequenc[ies] analysed against D_m EQD1 SKIN (D_{max}) a[nd] EQD1 VASC (D_{max}) Estimated values are based [on] a correction for local recurrence and death wit[hin] one year

Significant differences occur in the analysis [in] which the parameter is corrected for field si[ze]

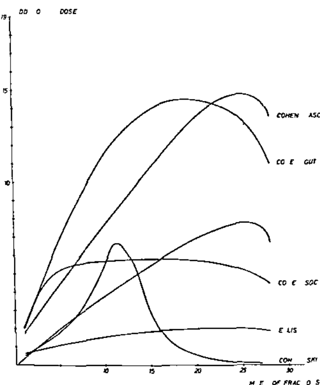


Fig 6 Calculations concerning split-course technique using different fractionation models First values are calculated for the schedule 60 Gy/30 fractions/41 days Then an interval of 30 days is inserted in the treatment period after N fractions The additional dose (Gy) means the dose that must be added to the last 30 N fractions delivered after the interval in order to obtain the values first calculated i e in order to obtain equal biologic effect in the split-course schedule and the continuous schedule (according to the model in question) The field size when needed is considered to be 10 cm Number of fractions (X axis) is the number of fractions delivered before the treatment interval

Thus (based on estimated frequencies) at EQD1 VASC=13 Gy (1/106=0 9 per cent 10/130=7 7 per cent p<0 025) and at EQD1 SKIN=17 Gy (0/134=0 0 per cent 11/102=10 8 per cent p<0 001)

The Cohen model for SKIN estimates the frequency of dry desquamative reaction as a function of EQD1 SKIN This estimate seems to be far from the observed frequencies of late edema The model for VASC predicts the risk of late effects in the vascular stroma (telangiectasis) and the risk seems to be approximately 20 per cent higher than the observed frequencies of late edema

Pharyngo-cutaneous fistula A total of 16 pharyngo-cutaneous fistulas have been observed all after total laryngectomy for local recurrence These are not clearly correlated to treatment level If the frequency of fistulas is calculated among the 72 patients on whom a total laryngectomy was performed a decreasing or constant frequency is found as function of D_c D_{max} and EQD1 SKIN (D_{ma}) while an increasing frequency occurs as function of EQD1 VASC (D_c) EQD1 VASC (D_{max}) and EQD1 SKIN (D_c)

Although it must be admitted that fistulas may develop for other reasons than previous irradiation an increasing risk with treatment level and field size should be expected The present material slightly indicates that the development of fistulas depends more on field size than on absorbed dose level The observed complications seems to be most satisfactorily related to the parameter EQD1 VASC

If fistulas necrosis and late edema are analysed together (which however should not be done) against the parameter EQD1 VASC (D_{max}) the frequencies are EQD1 VASC less than 12 GY (2/67~3 0 per cent) 12–14 Gy (11/138~8 0 per cent) 14–16 Gy (8/64~12 5 per cent) greater than 16 Gy (7/39~17 9 per cent) These frequencies are very similar to the estimated frequencies of late edema (Fig 5)

Working hypothesis The Cohen model for SQCA will correctly estimate the frequency of local recurrence The Cohen model for VASC will overestimate the frequency of late edema by 20 per cent

Optimum treatment level If the schedule is still restricted to 30 fractions 41 days an optimum value of absorbed dose may be estimated to be D ~65 Gy (D_{min}~62 Gy) This optimum level appears to be only slightly dependent on the actual combination of tumour size and field size By optimum is meant that the highest possible number of patients will pass the treatment without local recurrence or late edema Other criteria—such as to aim at the lowest number of total laryngectomies—might as well be justified

The effect of changing the absorbed dose from D =60 Gy to D =65 Gy is calculated to be a reduction in the frequency of local recurrence from 23 per cent to 13 per cent with an increase in the frequency of late edema from 10 per cent to 19 per cent (This is based on the distribution of tumour size and field size from the present material (Table 3) Although this result would represent an improvement there is evidence suggesting that the reduction in local recurrence may be obtained with no increase in late edema This result could possibly be achieved by the use of split-course technique

Conversion to split course schedule Several authors (HOLSTI 1967 1969a b 1971 SAMBROOK 1962 1963a b SCANLON 1960 1968) claim that an interval (usually of 2 to 4 weeks inserted after half

of the treatment period) will not affect the probability of local control if the absorbed dose is increased by approximately 10 per cent to compensate for tumour growth during the treatment interval and even then the patient will tolerate the treatment better The opinion of these authors is based on the treatment of several thousands of patients including some with laryngeal carcinoma

Fig 6 illustrates how the Cohen model for GUT VASC SKIN and SQCA the Kirk model (KIRK et coll 1975) and the Ellis model (ELLIS 1968 1969 WINSTON et coll 1969) would predict the effect of an interval of 30 days introduced in the treatment schedule 60 Gy/30 fractions/41 days

The Cohen model (SQCA) predicts that approximately a 10 per cent higher absorbed dose should be given in order to obtain equal probability of local control on squamous cell carcinoma This appears to be fairly independent of the position of the interval (and of tumour size)

As for the effect in normal tissue the Ellis model is not in accordance with the experience concerning the split-course technique the Kirk model is so to some degree whereas the Cohen model is so to a high degree with respect to the tissues in the gastrointestinal tract (GUT) and the vascular stroma (VASC) (Rectal complications have been analysed in 664 patients with carcinoma of the uterine cervix (HJELM HANSEN & SELL 1977) Excellent agreement with the Cohen model was found and the split-course technique based on the Cohen model has been adopted since January 1978 with good results) The Cohen model used for SKIN behaves differently and in fact (like the Ellis model) indicates that the frequency of complications increases in the split-course schedule Except when the parameter EQD1 SKIN is used the interval should preferably be placed in the last half of the treatment period

The opinions based on the split-course schedule mostly concern the acute reactions (patients tolerate the treatment better) while the Ellis model is based on late reactions However the Cohen model (VASC) is also based on late reactions (late effects in the vascular stroma such as telangiectasis or endarteritis) and the disagreement between the two models is an obvious matter for analysis

If the calculations are based on the working hypothesis and if the size of one fraction is restricted to the usual value (D_c=2 00 Gy) and the treatment interval to 21 days the optimum treatment (D_c=65 Gy/30 fractions/41 days) converted to split course technique becomes D_c=72 Gy/36 fractions/70 days with an interval of 21 days inserted after 20 fractions This schedule has been used during 1978 when the side of the equivalent square field did not exceed 8 cm When larger fields were used the schedule was reduced by 3 fractions

In the high level schedule the value of EQD1 SQCA will be greater than 22 Gy and the value of EQD1 VASC will be less than 12 Gy Thus according to the working hypothesis the frequency of local recurrence (Fig 2) should be less than 20 per cent (the overall frequency is calculated to 13%) while the frequency of late edema (Fig 5) should be very low since the previous experience (although without split-course) is 0/67 (confidence limits 0 00–7 60%)

However based on other parameters than EQD1 VASC different predictions are found indicating that the frequency of late edema may increase to as much as 30 per cent

Preliminary experience with the split course schedule From the experience gained during the first year (67 patients) the impression is that the patients tolerate the split-course schedule in approximately the same way as the old schedule i e acute effects are similar in spite of a considerable increase of absorbed dose The patients like the rest period and most patients report that any reaction felt at the beginning of the interval disappears during its course However slight reactions often reappear within the first week of continued treatment

It is too early for any statement about tumour response and late reactions However two surprisingly strong reactions have been observed (one required a total laryngectomy) and in order to reduce the risk it has been decided to use a reduced schedule from January 1979 (omitting the last two fractions) and await the analysis of local recurrence among the 67 patients with the high level split course schedule during 1978

Discussion

COHEN (1978b) discusses some of the limitations and possibilities of the model and the reader is referred to this discussion

The model (SQCA) has been tested on the present material and on 664 patients with carcinoma of the uterine cervix and although the analysis of this last material is much more complex the same tendency

of excellent agreement with the model was found In both materials the model is predicting slightly lower frequencies of local recurrence than observed Since the present data for high values of EQDI SQCA (>22 Gy) would justify only a slight adjustment of he model and since this part of the response curve is the important part with respect to optimization no adjustment will be proposed at this stage The model has only been tested on schedules with 5 fractions per week with only a few (and small) unplanned intervals

The optimum schedule calculated in the present analysis could (according to the model) be better if the restriction of 5 fractions per week could be dis regarded Comparison with results from the litera ture is difficult because information about tumour size is lacking However at least a qualitative com parison could be made with two randomized trials comparing 5 fractions per week with 3 fractions and 1 fraction per week respectively

One of these is the trial run by the British Institute of Radiology since 1963 of 5 versus 3 fractions per week in a number of different schedules (Second Progress Report 1968) In the Sixth Interim Progress Report (1978) on this test it is reported that among 687 patients treated those who received 5 fractions per week have a slightly higher (~5%) local recur rence free rate

According to the Cohen model some of the schedules used (long overall time) would be better (5–10%) with 5 fractions per week while others would be equal or slightly better (0–3%) with 3 fractions per week Thus it is in excellent agreement with the model that only a small difference has been observed The largest difference has been observed among the smallest tumours (cords mobile) but it should be noted that any odd distribution of tumour size in the two groups would give rise to a much larger difference than the one caused by the fractionation According to the model the schedules with 5 fractions per week should give rise to more (5 to 10%) complications which however have not been observed

The second trial of 5 versus 1 fraction per week was presented by DVIVEDI et coll (1978) and in cludes 341 patients among which 202 patients were head and neck cases Although the follow up is in complete and short the authors conclude that the tumour response is equal and that 1 fraction per week is tolerated better The schedules in question were 30×2 00 Gy/41 days and 6×6 70 Gy/36 days

The Cohen model predicts that equal tumour re sponse would require a weekly fraction of 6 60 Gy and that the weekly fractionation should be as sociated with a 14 per cent lower complication rate

Thus it seems to be indicated that the Cohen mod el may also be used on schedules with 3 and 1 fraction per week

At the time of writing data from August 1972 to December 1977 are being collected (60 Gy/30 frac tions/41 days) and although the follow up on some of these patients is short it may already be stated that it is very unlikely that addition of these data will cause a change in the working hypothesis based on the Cohen model

SUMMARY

Long term results obtained in the treatment of 308 patients with laryngeal carcinoma are analysed with re spect to local recurrence and complications In this analysis the Cell Population Kinetic model proposed by COHEN (1971) is used The model for squamous cell car cinoma is found to be useful and in close agreement with observations of local recurrence although a slight modi fication may be justified from the present data No special model exists for late edema but the model for late effects in the vascular stroma seems to be useful in this respect Based on the model a working hypothesis has been proposed by which it is possible to predict the frequency of local recurrence and late edema An optimum treatment schedule is calculated and converted to a split-course technique

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FROM INSTITUTO DE ONCOLOGÍA Y RADIOBIOLOGÍA LA HABANA HOSPITAL ONCOLÓGICO DOCENTE SANTIAGO DE CUBA AND HOSPITAL ONCOLÓGICO CAMAGUEY CUBA

COMPLICATIONS FROM IRRADIATION OF CARCINOMA OF THE UTERINE CERVIX

J ALERT J JIMENEZ L BELDARRAIN J MONTALVO and C ROCA

Cervical carcinoma is the second most frequent malignant neoplasm in Cuban women with an annual average of about 650 new patients and a crude incidence rate of 16 1 per 100000 and year (Registro Nacional del Cancer 1975) Irradiation is the principal method used with a combination of intracavitary and external irradiation Its use implies a certain risk of complications from organs situated within the irradiated volume the intestine and bladder being the organs most often affected

The types and the frequency of complications in a group of patients treated by irradiation alone are now reported

Material and Methods

The material consisted of 2248 patients with carcinoma of the uterine cervix who were irradiated between 1966 and 1972 at three oncologic centers 1280 (56 9%) at Instituto de Oncologia (Havana City) 803 (35 8%) at Hospital Oncologico Docente (Santiago de Cuba) and 165 (7 3%) at Hospital Oncologico (Camaguey) The method of treatment and the results have been reported previously (JIMENEZ et coll 1979)

Complications were classified as early when the symptoms and signs appeared during the irradiation series or within 6 months from its completion and as late when they appeared 6 months or later after the end of irradiation Cases where malignant tissue was found in the walls of fistulas were excluded

Results

Complications were recorded in 458 patients (20 4%) The total number was 523 as some patients had more than one complication (Table) Of these 305 (58 3%) were classified as early and 218 (41 7%) as late

Proctitis was the most frequent complication and occurred in 15 5 per cent of the total number of patients The degree of proctitis varied from only tenesmus and edema of the rectal wall to ulceration and bleeding During or at the end of irradiation some patients developed acute symptoms such as tenesmus painful defecation and diarrhea which were classified as early proctitis Late proctitis usually appeared between six months and one year from the end of irradiation In 176 patients late proctitis occurred and in all but 10 this was preceded by early proctitis

More severe complications as fistulas ureteral stenosis with hydronephrosis enterocolitis sigmoiditis intestinal obstruction and skin necrosis occurred in 81 patients (3 6%) fistulas developed only in 36 patients (1 6%)

Colostomy was performed in 11 patients (0 5%) for the following reasons severe proctitis with ulceration of the rectal wall and uncontrollable bleeding (3 patients) fistulas (3 patients) and intestinal obstruction (5 patients)

A similar incidence of complications occurred af

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Table

Type and frequency of complications following irradiation

Type of complication	Number	Per cent
Proctitis	348	66 5
Cystitis	74	14 1
Recto-vaginal fistula	28	5 3
Ureteral stenosis with hydronephrosis	24	4 6
Panniculitis	17	3 3
Intestinal obstruction	13	2 5
Vesico-vaginal fistula	8	1 5
Sigmoiditis	4	0 8
Edema of legs	3	0 6
Skin necrosis	2	0 4
Enterocolitis	1	0 2
Fistula between sigmoid and jejunum	1	0 2

ter the two principal techniques used external irradiation followed by intracavitary radium applications or intracavitary radium applications followed by external irradiation

Discussion

Radiation therapy similar to surgery or cytostatic therapy implies certain risks of complication The literature reporting and discussing the incidence and types of complications is extensive (GRAY & KOTTMEIER 1957 FLETCHER et coll 1958 CHAU et coll 1962 CASTRO et coll 1970 STROCKBINE et coll 1970 BORONOW & RUTLEDGE 1971 FLETCHER 1971 JOELSSON et coll 1971 SLATER & FLETCHER 1971 MICKAL et coll 1972 ROSWIT et coll 1972 VILLASANTA 1972 FRIBERG & JOHNSSON 1974 MARUYAMA et coll 1974 CHISM et coll 1975 EINHORN 1975 JOHNSSON 1976 MARCIAL 1977 PEREZ et coll 1977 ALERT 1978 ALERT et coll 1978) The complications are caused by the radiation dose to different parts of the small pelvis and the lower part of the abdomen which causes interstitial edema necrosis and local fibrosis Individual variations in sensitivity and the pretreatment condition (general condition local inflammatory diseases) certainly also play a role

In practice the distribution of the dose can deviate considerably from the calculated ideal dosimetry due to different clinical factors distorting the geometry of the small pelvis (SCHWARZ 1969) The determination of the doses at points in the paracervical region with regard to the intracavitary radium arrangement can be a dangerous practi Both with inverted T and the linear arrangement o the radiation sources a decrease in the dose m appear from the surface of the applicators to th periphery of the small pelvis This decrease is in enced by different factors such as the distance be tween the vaginal sources the length of the line arrangement etc (FLETCHER ALERT) Vagi applicators situated preferentially in the posteri vault increase the dose in the rectovaginal wall a increase the risk of proctitis

Although 348 patients had proctitis colostom was needed only in 3 Rectovaginal fistulas occur in 28 patients an incidence of 1 2 per cent

External irradiation before radium applicati causes high doses to large parts of the intestine B the external irradiation the thickness of the uteri wall may also be reduced which may cause a highe intestinal dose from the intracavitary irradiation I seems likely that both these factors contribute to th intestinal complications found (EINHORN)

Sigmoiditis has been reported as a specific com plication of whole pelvis irradiation especially afte external irradiation alone (FLETCHER) The patient with a fistula between the sigmoid and jejunum in the present series had received external and in tracavitary irradiation Probably the uterine fundu was situated close to the sigmoid with a resulting high local dose

Ureteral strictures which have been reporte to occur with a very low incidence (SLATER & FLETCHER) were observed in 24 patients The sig of obstruction varied from slight or moderate dila tion of the ureter and renal pelvis to complete lack o kidney function on the obstructed side

The total observed incidence of severe complica tions was in the present series 3 6 per cent Only 0 5 per cent of the patients was surgical treatment of complications required

The risk of complications in connection wi radiation therapy may be reduced to some extent b careful dose planning and continuous observation o the patient during the treatment However it mus be emphasized that a certain frequency of complica tions must always occur as a consequence of irradia tion

SUMMARY

The types and frequency of complications in 2 48 pa tients irradiated for carcinoma of the cervix using differe techniques are reported Proctitis was the most freque

complication Severe complications such as fistulas ureteral stenosis with hydronephrosis enterocolitis sigmoiditis intestinal obstruction and skin necrosis occurred in 81 patients (3 6%) Only 36 patients (1 6%) developed fistulas

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CHROMOSOME COUNTS OF ^{90}SR INDUCED OSTEOSARCOMAS IN MICE

I. Transplanted tumour series

H. BERGMAN and A. NILSSON

Hypotheses concerning radiation induced malignant tumours which for the present are consonant with available data are those concerning initiating and promoting events, i.e. a multi step mechanism (NILSSON 1975) supposed to be somatic mutations. As the present knowledge of chromosome injury is limited, and as many radionuclides are extremely potent carcinogens, cytogenetic investigations should contribute to an increased understanding of the induction mechanism. For this purpose ^{90}Sr induced tumours should constitute a suitable experimental model, as the development of these tumours (NILSSON 1966), their histologic type (NILSSON 1962), dose dependence (NILSSON 1969) and several other characteristics have been clarified in detail. The main purpose of the present investigation was to form a conception of the chromosome numbers of the primary tumours and especially the late chromosomal progression by serial in vivo transplantation. An intention was also to analyse whether additional and predetermined steps could be detected, as found in serially transplanted Rous rat sarcomas (MITELMAN 1972). A comparison was made between the chromosome pattern of fast and slow growing tumour series (Parts II and IV, BERGMAN 1980) and whether transplantation to mice with a changed immunologic status is combined with a modified chromosome distribution (Part II). A comparison was also performed on serially in vivo transplanted tumours and parallelly in vitro cultured tumour cells (Part III, BERGMAN 1980). The chromosome pattern of early ^{90}Sr induced primary tumours, so called tumour buds, will later be investigated in order to ascertain whether chromosome aberrations can be proved at these relatively early stages of the tumour evolution.

Material and Methods

Inbred, about 60-day old female mice of the CBA strain were used. Primary osteosarcomas were induced by intravenous injection of 14.8 to 29.6 kBq $^{90}Sr(NO_3)_2$ per g body weight (Table 1). Tumours were localised by radiography 288 to 403 days after the injection of ^{90}Sr (Fig. 1). Tumour bearing mice were killed by cervical dislocation. Peripheral and homogeneous parts of the tumours were placed in physiologic saline and pinhead sized pieces were subcutaneously transplanted to the neck of 10 male mice. From these mice a succeeding generation was obtained by transplantation from one of the outgrown tumours, to another 10 mice. A diagram of the transplantations is presented in Fig. 2. By this type of serial transplantation 3 transfer series, A to C, were established from 3 primary tumours. The number of transfer generations and tumours examined is given in Tables 2, 4 and 6. Samples were taken from the primary tumours, as well as from the tumours used for transplantation, for chromosome analysis. After an outgrowth period of 10 to 72 days the tumour volume was calculated by using a caliper. The tumours were carefully dissected and all necrotic parts were discarded. Small pieces of the tumours were prepared by mincing through a steel wire gauze. The material was incubated at 38°C in a 1% sodium-citrate solution for 15 min and centrifuged at 800 rpm for 10 min. The supernatant was pipetted off and the cell suspension was fixed in glacial acetic acid and ethanol (1:3) for 30 min. The fixation procedure was repeated 2 to 3 times. The preparations were air dried and the cells

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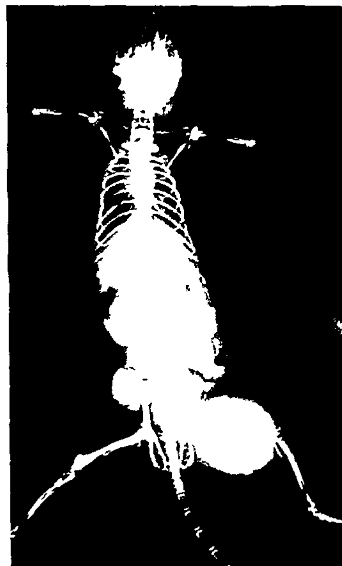


Fig 1 Primary osteosarcoma A in the left femur 288 days after the injection of ^{90}Sr

stained in acetic orcein (2% orcein in 60% acetic acid) for 1 h or according to the conventional Giemsa method The preparations were observed in a Wild Heerburg M 20 microscope Cells suitable for chromosome counting were selected at a magnifica

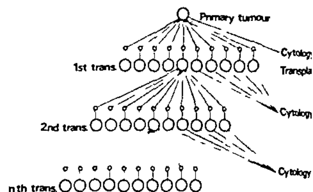


Fig 2 Diagram of transplantations

tion of 200× and analysed at 1000× Only appare ly well spread and unbroken metaphases were cl sen At an early stage an optimum number of w spread metaphases per tumour were examin However due to poor preparations it later beca necessary to decrease the number of cells to 25 even below this level At the most 5 tumours p transfer generation were analysed but only trans generations from which it was possible to analyse least 2 tumours are presented Due to the fe chromosome counts per tumour it was preferred refer to the chromosome counts per transfer gene tion It should also be noted that from transf generation 25 and on analysis was performed on sporadically

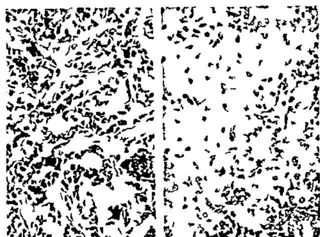
For histologic examination sections of the p mary tumours and a selection of tumours along t transfer series were fixed in Stieve s solution e bedded and stained according to the van Giesc method or with haematoxylin eosin

Definition of tumour classification The osteosa comas were subdivided into fibroblastic chondr blastic osteoblastic or osteoclastic types In add tion pleomorphic mixed and anaplastic types we distinguished depending upon whether a strong pleomorphic cell type equal parts of at least thre

Table 1

Different characteristics of the primary osteosarcomas

Primary tumour	Dose ^{90}Sr (kBq/g body weight)	Induction time (days)	Site of the tumour in the skeleton	No of cells analysed	Chromosome number									
					35	36	37	38	39	40	41	42	61	72?
A	29 6	288	L femur	26	1	1	1	10	7	3		1		
B	14 8	403	L tibia-femur	–										
C	14 8	403	R femur	17					2	10	3	1	1	





ig 3 Primary osteosarcoma B from a mouse given 14 8 kBq ⁹⁰Sr/g body weight appearing 403 days after the injection Distinct bone (a) and cartilage (b) formation but with predominance of low differentiated and pleomorphic mainly fibroblastic tissue (c) infiltrating surrounding muscles Abundancy of mitosis Haematoxylin eosin ×240

cell components or a low differentiated tissue predominated This subdivision has been recommended by the Committee of Pathology of the European Late Effects Project Group (EULEP 1971)

Results

Histology The primary tumour A was characterized as a predominantly fibroblastic osteosarcoma with a slight osteoid formation Primary tumour B

Table 2

Transfer series A Different characteristics of the transfer generations examined

Transfer generation	No of tumours examined	No of cells counted	Mean growth period (days)	Mean size (cm³)	Mean growth per day (cm)	Pre dominating chromosome No	Per cent	Percentage of cells with metacentrics
2	5	108	19 2	0 88	0 046	38	67 1	5 2
3	5	164	17 0	1 00	0 059	38	51 2	6 0
4	4	173	18 8	1 03	0 055	38	50 9	5 9
5	9	208	18 1	1 16	0 064	38	36 5	4 6
6	3	85	21 3	0 89	0 042	37	40 0	5 9
7	6	272	24 3	1 03	0 042	37	47 4	3 9
8	3	13[illegible]	24 3	0 73	0 030	37	43 2	9 8
9	4	101	19 5	0 94	0 048	37	59 4	5 9
10	3	75	[illegible]0 3	1 07	0 053	37	48 0	10 0
1[illegible]	6	150	16 5	0 79	0 048	37	31 3	5 3
13	2	50	6 0	1 49	0 057	37	48 3	16 0
14	4	95	[illegible]0 5	0 88	0 043	37	48 4	0
16	3	75	19 0	1 12	0 059	37	34 7	2 7
17	7	161	19 9	1 03	0 052	37	36 0	3 1
0	3	75	15 3	0 87	0 057	40	84 0	0
25	3	75	14 7	0 95	0 065	40	90 7	0
43	3	75	15 0	0 75	0 050	40	66 7	1 3
45	3	75	15 7	0 84	0 054	40	41 3	1 3
48	4	100	9 8	0 35	0 036	40	71 0	1 0
50	3	75	14 0	1 56	0 111	40	46 7	0
53	3	75	11 3	0 66	0 058	40	66 7	0
54	5	125	16 0	0 99	0 062	40	64 0	13 5
55	4	100	15 0	0 79	0 053	40	70 0	14 0

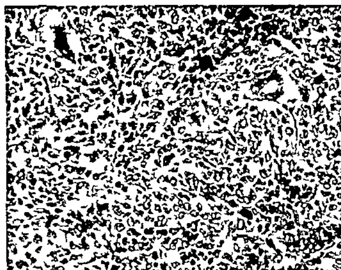


Fig 4

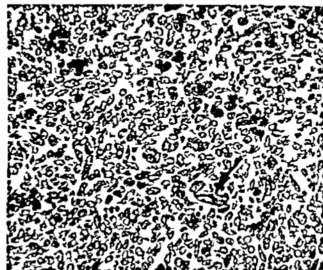


Fig 5a



Fig 5b

Fig 4 From transfer series A Highly anaplastic tumour from the 22nd transfer generation numerous giant cells van Gieson ×240

Fig 5 a) From transfer series C Highly anaplastic osteosarcoma from a tumour of transfer generation 2 Tumour bone (→) van Gieson ×240 b) Tumour from transfer generation 18 Area with abundance of giant cells and tumour bone van Gieson ×240

Table 3

Transfer series A The percentile chromosome distribution of 11 transfer generations

Transfer generation	No of tumours examined	No of cells counted	Chromosome number 32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	4[illegible]	[illegible]
2	5	108		0 9			1 9	8 3	61 1	13 0	3 7	1 9		0 9					
5	9	08	0 5		3 4	1 9	1 5	29 8	36 5	7 2	0 5	1 4	0 5		0 5				0 5
6	3	85	1 2		2 4	4 7	11 8	40 0	15 3	15 3	1 2		1 2						
9	4	101				2 0	12 9	59 4	11 9	2 0	3 9					1 0			
17	7	161			1 9	7 4	8 1	36 0	8 7	4 9	24 8	0 6							
25	3	75						1 3		2 7	90 7	2 7							
43	3	75									66 7		1 3						
45	3	75									41 3		1 3						1[illegible]
50	3	75									46 7		1 3		1 3				1[illegible]
54	5	125							0 8	0 8	64 0					0 8			
55	4	100								3 0	70 0		1 0						

Fig 6 A 38-chromosome cell from a tumour of transfer series A generation 4

Fig 7 A 37-chromosome cell from a tumour of transfer series A generation 5

was classified as an osteosarcoma of predominantly mixed type containing cartilage components of varying degree of differentiation plus tumour bone and peripherally mainly cells of anaplastic type (Fig 3) After consecutive transplantations these tumours were successively transformed into more and more anaplastic entities (Figs 4 5) Finally primary tumour C and the serially transplanted tumours were characterized as osteosarcomas of predominantly anaplastic type

Chromosome analysis The mouse has normally 40 chromosomes per cell The registration of abnormalities was limited to numerical chromosome deviations and the occurrence of metacentric configurations For good surveyability of the extensive material the presentation of the chromosome distribution is concentrated to the main findings For this reason primarily transfer generations reflecting more marked changes in the chromosome distribution are shown Furthermore only regions within which the major chromosome distribution was found are presented

Transfer series A The primary tumour and 95 transplanted tumours of transfer series A were

Table 3 *(cont)*

51	5	53	54	55	56	57	58	59	60	61	62	71	7?	73	74	75	76	77	78	79	80
												0 9	0 9	1 9		0 9	8			0 9	
												0 5	1 4	0 5	1 0	0 5	1 0				
															4 7	1.	1.				
											1 0			3 0	2 0		1 0				
												0 6	1 9	1-	1 9	0 6	1-				
									1 3												1.3
						1 3		3	8 0	1 3											
			1 3	?7			5	5.3	8 0	10 7	4 0										
		1.3	1 3					1 3	16 0	-6 7	2 7										
3	6 4	17 6	4 8	0 8																	
	4 0	11 0	7 0	4 0																	

Table 4

Transfer series B Different characteristics of the transfer generations

Transfer generation	No of tumours examined	No of cells counted	Mean growth period (days)	Mean size (cm³)	Mean growth per day (cm³)	Pre dominating chromo-some No	Per cent	Percentage of cells with metacentrics
1	5	125	20 2	0 79	0 039	40	65 6	0
2	5	125	25 0	1 43	0 057	40	25 6	37 0
3	5	125	18 0	0 93	0 052	60	50 4	5 6
4	5	125	17 7	1 14	0 064	40	51 2	0 8
5	5	125	18 6	1 42	0 076	40	33 6	2 4
6	5	125	13 8	1 03	0 075	40	73 6	0 8
7	5	125	14 0	1 26	0 090	40	39 2	2 4
8	5	125	14 2	1 72	0 121	40	55 2	0
9	5	125	13 0	1 41	0 108	40	50 4	1 6
10	5	125	13 0	1 38	0 106	40	63 2	0
11	5	125	15 4	0 84	0 055	40	86 4	0
12	5	125	14 6	1 50	0 103	40	72 8	0 8
13	5	125	16 0	1 23	0 077	40	67 2	0
14	5	125	13 4	0 93	0 069	40	56 0	0
15	4	100	14 3	1 06	0 074	40	41 0	0
16	4	99	15 8	1 24	0 078	61	40 4	0
17	5	125	15 0	1 06	0 071	40	36 0	0 8
18	5	125	14 4	0 73	0 051	40	52 0	0
19	3	75	18 7	0 83	0 044	60	36 0	1 3
20	5	125	16 0	1 05	0 066	40	48 0	0 8
21	5	125	16 6	1 20	0 072	40	60 0	0
22	4	100	15 3	1 29	0 084	40	56 0	0
23	5	125	12 0	0 94	0 078	40	59 2	0 8
25	3	75	14 3	1 09	0 076	40	61 3	0
34	4	100	13 0	0 75	0 058	40	62 0	0

Table 5

Transfer series B The percentile chromosome distribution of 10 transfer generations

Transfer genera tion	No of tumours examined	No of cells counted	Chromosome number																	
			39	40	41	42	43	53	54	55	56	57	58	59	60	61	62	63	64	65
1	5	125		65 6	1 6							1 6	1 6	3 2	2 4	4 0	5 6	8 8	4 8	0 8
3	5	125	2 4	22 4				0 8				3 2	6 4	6 4	50 4	4 8	1 6	1 6		
6	5	125		73 6												0 8	12 0	12 8	0 8	
7	5	125		39 2									0 8	2 4	8 0	16 8	21 6	8 8	2 4	
11	5	125	0 8	86 4	1 6				0 8			0 8	0 8		1 6	4 8	2 4			
16	4	99	1 0	16 2							2 0		1 0	4 0	24 2	40 4	10 1	1 0		
19	3	75		18 7	1 3							5 3	5 3	25 3	36 0	5 3	2 7			
20	5	125		48 0					1 6				1 6	8 0	33 6	4 8	0 8	1 6		
25	3	75	1 3	61 3				1 3			1 3	1 3	6 7	6 7	13 3	6 7				
34	4	100		62 0			1 0				1 0	4 0	20 0	10 0	1 0					

examined with regard to chromosome pattern in 55 transfer generations The percentile chromosome distribution from 11 selected generations is pre sented in Table 3 The chromosome counts of the primary tumour are given in Table 1 and different characteristics of the transfer generations in Table 2 The primary tumours as well as the early transfer generation (2–4) were mainly characterized by a predominance of 38 chromosome cells (Fig 6) but often with a wide range of variation With the fifth

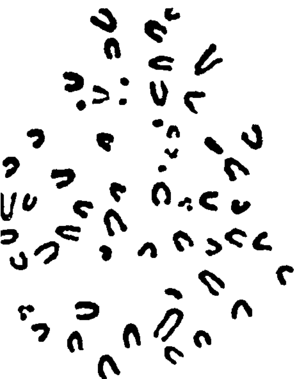


Fig 8 A 56-chromosome cell from a tumour of transfer series B generation 4

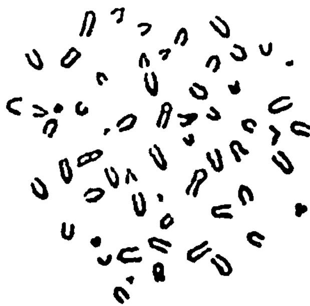


Fig 9 A 60-chromosome cell from a tumour of transfer series B generation 4

Table 6

Transfer series C Different characteristics of the transfer generations examined

Transfer generation	No of tumours *examined*	No of cells *counted*	Mean growth *period* (days)	Mean size (cm^3)	Mean growth per day (cm^3)	Pre dominating chromo some No	Per cent	Percentage of cells with metacentrics
2	5	125	36 0	0 47	0 013	40	97 6	0 8
3	5	173	24 4	1 33	0 055	40	94 3	0 8
4	2	50	32 0	0 71	0 022	40	98 0	0
5	5	125	24 0	0 66	0 078	40	95 2	1 6
6	5	172	30 4	1 14	0 038	40	91 0	3 3
7	5	124	20 8	0 84	0 040	40	89 5	0
8	4	96	18 0	0 80	0 044	40	58 3	25 0
9	2	50	20 0	0 60	0 030	40	96 0	0
10	3	75	13 0	0 78	0 060	40	100 0	0
11	2	50	31 0	0 60	0 019	40	96 0	2 0
12	3	75	23 7	0 88	0 037	40	98 7	0
13	3	75	17 0	1 04	0 061	40	100 0	0
18	3	75	6 0	0 68	0 0 6	40	90 7	5 3
19	5	125	17 4	0 86	0 049	40	72 0	16 2
20	5	125	20 0	1 14	0 057	40	78 4	7 0
21	4	100	5 3	0 67	0 044	40	47 0	0
22	5	125	1 6	1 30	0 103	40	64 0	0
23	3	75	9 3	0 68	0 073	40	92 0	0
24	3	75	11 0	0 86	0 078	40	78 7	0

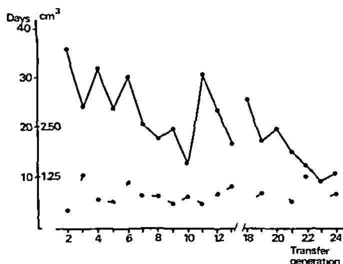


Fig 10 Solid curve Variation in mean outgrowth period of the tumours per transfer generation Interrupted line Mean size of the tumours per transfer generation The example is from the C series

transfer generation an increased number of 37 chromosome cells (Fig 7) appeared This predominance of 37 chromosome cells was persistent from generation 6 to 17 Well spread preparations were now not obtained until transfer generation 20 However from there up to the 25th generation a modified chromosome pattern with a majority of 40 chromosome cells and only small numbers of 37 and 38-chromosome cells was found Beyond this generation tumour analysis was only sporadically performed which means that the development of the chromosome pattern in the successive generations is uncertain An analysis of the tumours of transfer generation 43 revealed a disparate chromosome distribution Here the 40-chromosome cells were still predominant but in addition cells with chromosome numbers ranging from 57 to 61 were fo Except for percentile variations it was found the split chromosome distribution was maint also in transfer generation 45 48 50 and 53 F ly generations 54 and 55 showed a shift from 5 61 chromosome cells to cells with chromo numbers varying from 50 to 55 but still w majority of 40-chromosome cells

Transfer series B In this series 34 transfer ge tions were established Chromosome counts obtained from 117 tumours The chromosome tributions of 10 transfer generations are present Table 5 while Table 4 shows different charac tics of the transfer generations examined Excep generations 2 16 and 17 these series show predominance of 40 chromosome cells plus o sionally an altered chromosomal pattern withi triploid region The percentage of 40-chromos cells varied within the series from 16 2 to 86 4 t last examined transfer generations showing n 60 per cent of 40-chromosome cells Concernin triploid region the early transfer generation displayed a wide distribution of chromos numbers ranging from 54 to 65 but with a pred nance of 60 62 and 63-chromosome cells F shows a 56 chromosome cell and Fig 9 a chromosome cell from a tumour of transfer eration 4 The subsequent transfer generations showed a range of 57 to 64 and a noticeably frequency of 60 to 64-chromosome cells p observed at 61 and 62 Transfer generations 10 displayed a preponderant number of 60- and chromosome cells while transfer generations 25 were characterized by a preponderance of chromosome cells but also by an increased nun

Table 7

Transfer series C The percentile chromosome distribution of 9 transfer generations

Transfer generation	No of tumours examined	No of cells counted	Chromosome number 39	40	41	42	47	50	51	52	53	54	55	56	57	58	59
2	5	125	1 6	97 6													
5	5	125	2 4	95 2								0 8		1 6			
8	4	96	1 0	58 3													
10	3	75		100 0													
13	3	75		100 0													
19	5	125		7[illegible] 0		0 8	0 8	0 8	0 8	4 0	13 6	4 8	0 8				
20	5	125	3 2	78 4						4 0	11 2	1 6	0 8				
22	5	125	[illegible] 4	64 0	1 6				0 8					0 8		4 6	
[illegible]4	3	75		78 7									1 3	1 3	4 0	10 7	

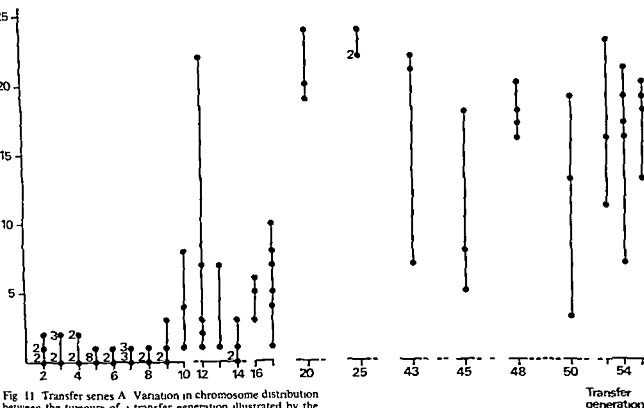


Fig 11 Transfer series A Variation in chromosome distribution between the tumours of a transfer generation illustrated by the number of 40-chromosome cells per tumour (●) The figures at the tumour symbol indicate that two or more tumours had the same number of 40-chromosome cells

of cells within the 57 to 59 region Transfer generation 34 which was the last examined generation displayed within the triploid region a majority of 58 chromosome cells and a range of 55 to 60

Transfer series C In transfer series C the primary tumour and 72 tumours of 24 transfer generations were examined Table 7 shows the percentile chromosome distribution of 9 transfer generations

Table 7 *(cont)*

1	6	63	64	65	66	69	80
						0 8	
6 3	7 3	8 3	13 5	[illegible]	2 1		
							6
0 8							
1	4 0	0 8					0 8
1 3							

The few chromosome counts obtained from the primary tumour are presented in Table 1 and different characteristics of the transfer generations examined in Table 6 The primary tumour showed a narrow range of variation around 40 Except for transfer generation 8 the tumours of generations 1 to 18 contained a predominance of 40-chromosome cells ranging from 89 5 to 100 per cent In transfer generations 19 to 24 a lower percentage of 40-chromosome cells was recorded and instead a major range of 50 to 55 and 55 to 63 for the generations 19 to 20 and 21 to 24 respectively In this context it should also be mentioned that a large number of consecutive transplantations of tumours generally led to an increased rate of growth of the transplanted tumours (Fig 10) Furthermore the variation in chromosome distribution between tumours of one and the same transfer generation is illustrated by the number of 40-chromosome cells per tumour and generation in Figs 11 to 13

Metacentric chromosomes The staining method used does not permit a detailed and adequate analysis of the karyotypes However the technique

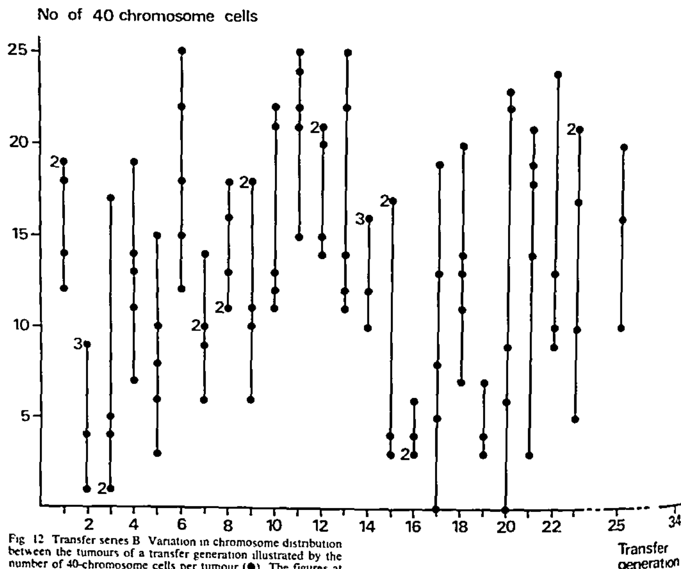


Fig 12 Transfer series B Variation in chromosome distribution between the tumours of a transfer generation illustrated by the number of 40-chromosome cells per tumour (●) The figures at the tumour symbol indicate that two or more tumours had the same number of 40-chromosome cells

has revealed evidence of chromosome breakage and reunion through observation of extremely long chromosomes minutes and metacentrics The majority of these gross structural abnormalities appeared quite temporarily As mouse chromosomes are normally telocentric metacentric configurations are easily recognized The percentage of cells with one or more metacentrics per transfer generation appear in Tables 2 4 and 6 Even if these aberrations as well occurred quite randomly and none seemed particularly well adapted they occasionally constituted recurrent events

Conclusions

Whether an unbalanced karyotype may be causally related to malignancy is a basic problem that chromosome analyses on tumours are attempting to solve As numerous radionuclides are extremely potent carcinogens cytogenetic investigations of radiation induced malignant tumours should contribute to an increased understanding of the induction mechanism However the primary intention of the present investigation of ^{90}Sr induced osteosarcomas was to obtain a conception of the chromosome pattern in late phases of the tumour progression and to examine whether additional or pre determined steps could be detected This was made possible by serial transplantation from the three primary tumours used Concerning the early transfer generations they displayed different chromosome appearances (Tables 3 5 and 7) Thus it was found that the A series was characterized by a predominating number of 38 and 37-chromosome cells the B-

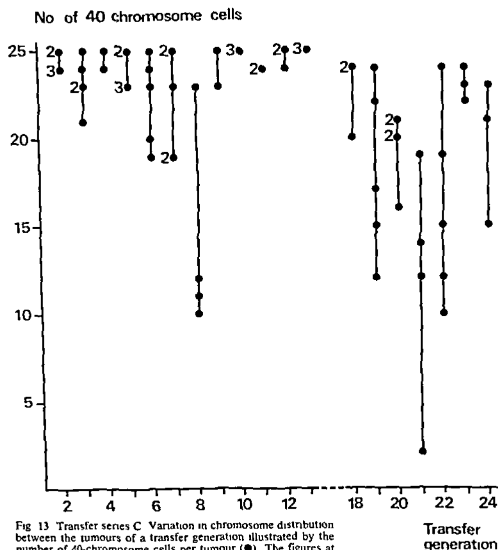


Fig 13 Transfer series C Variation in chromosome distribution between the tumours of a transfer generation illustrated by the number of 40-chromosome cells per tumour (●) The figures at the tumour symbol indicate that two or more tumours had the same number of 40-chromosome cells

series by 40 chromosome cells in combination with cells within the 52 to 64 region while the tumours of the C series displayed a distinct dominance of 40-chromosome cells Of special interest was that in spite of this heterogeneous development striking similarities appeared but during different stages of the tumour evolution These similarities in chromosome pattern between the three transfer series consisted of a predominant number of 40 chromosome cells in combination with cells within the 52 to 64 region This chromosome distribution in the B series was observed already in the first transfer generation and with minor exceptions was maintained along the whole series In the C series this type was not established until transfer generation 18 and in the A series not until transfer generation 43 However when this special distribution appeared and except for a certain variation from tumour to tumour it seemed well balanced

SUMMARY

From ⁹⁰Sr induced primary tumours three transfer series were established by serial in vivo transplantation Chromosome counts were obtained from 2 of the primary tumours and 284 transplanted tumours The recording of abnormalities was limited to numerical chromosome deviations and the occurrence of metacentric configurations By means of the serial tumour transplantation the numerical chromosome progression was also analysed Though appearing at different stages of the tumour evolution similarities in chromosome pattern were observed

ACKNOWLEDGEMENT

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EFFECT OF SYNGENEIC BONE MARROW AND THYMUS CELL TRANSPLANTATION TO ^{90}Sr IRRADIATED MICE

A NILSSON P BIERKE and A BROOMÉ KARLSSON

It is a wellknown fact that the acute haematologic risis induced by supralethal irradiation doses may e restored by transplantation of allogeneic or enogeneic bone marrow cells whereas syngeneic one marrow cells are a necessity when midlethal radiation doses are used These problems have een analyzed in several reports as regards external whole body irradiations (JACOBSON et coll 1951 COLE et coll 1955 CONGDON et coll 1957 URSO & CONGDON 1957 UNSGAARD 1961 DUPLAN et coll 962 BARNES et coll 1966 JARPLID 1968 COVELLI t coll 1974 METALLI et coll 1976)

Syngeneic bone marrow cells injected to sup ralethally irradiated mice will according to COVELLI et coll enhance the short term survival of the ir radiated animals On the other hand the life span of the survivors is reduced mainly on account of an increased incidence of degenerative and neoplastic diseases It has been shown by KAPLAN & BROWN (1952) KAPLAN et coll (1953) WALLIS et coll (1966) and JARPLID (1968) that the incidence of lym phoreticular tumours (LR tumours) may be consid erably reduced by shielding parts of the bone mar row during irradiation or by injection of bone mar row cells within a certain interval after whole body irradiation

To which extent these modifications also are valid after contamination with nuclides do not seem to have been of much interest It has been well docu mented (NILSSON 1962 1970 NILSSON & BROOMÉ KARLSSON 1976 JÄRPLID 1973) that bone seeking internal emitters may have a very destructive effect upon the haematopoietic system and of immunologi cally potent cells as well (NILSSON 1970 JARPLID 1973 HALLER & WIGZELL 1977) Such animals will develop signs of a bone marrow aplasia such as general anaemia haemorrhagic diathesis and mal functions of the specific and unspecific defence sys tem of the body as well as a high incidence of neoplasia mainly osteosarcomas and LR tumours

The aim of the present report was to investigate if syngeneic bone marrow cells or thymic cells given to ^{90}Sr irradiated mice could influence upon factors such as life span tumour incidence and tumour in duction time of mainly osteosarcomas and LR tumours

Materials and Methods

The animals were divided into two experimental series designed A and B containing 200 female and 250 male CBA mice respectively (Table 1) Carrier free ^{90}Sr (NO_3) was given to each of the series intraperitoneally at the age of 75±5 days The sub division of the main series into groups (I–VIII) and their irradiation with ^{90}Sr and treatment with sus pensions of bone marrow and thymic cells are recorded in Table 1 Since the bone marrow cavity is a very hostile environment for invading transplanted cells particularly during the first few months after ^{90}Sr injection two dose levels 25 9 and 14 8 kBq (0 7 and 0 4 μCi)/g body weight were chosen in order to differentiate the radiation milieu for the homing

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Table 1

Age of the mice at start of the experiment 75±5 days Day 0 Day of $^{90}Sr(NO_3)_2$ administration Intravenous dose of bone (BM) cells 5×10⁶ cells Intravenous dose of thymic (TH) cells 5×10⁶ cells

Experiment	Group of mice	No of mice	Sex	Dose of ^{90}Sr/g b w		Cell transplantation (days after ^{90}Sr inj)			
				μCi	kBq	BM-cells		TH cells	
A	I	50	F	0 7	25 9	–			
	II	50	F	0 7	25 9	30 60 90	210		
	III	100	F	0 7	25 9	30 60 90	210	30 60 90	210
B	IV	50	M	0 0		–			
	V	50	M	0 4	14 8	–			
	VI	50	M	0 4	14 8	1 30 60 90	Every month		
	VII	50	M	0 4	14 8	–		1 30 60 90	Every mont
	VIII	50	M	0 4	14 8	1 30 60 90	Every month	1 30 60 90	Every mont

Every month The mice were given cell transplants monthly during their whole life span
Eighteen animals lost at the cell transfusion
Three animals lost
One animal lost
Five animals lost

Table 2

Frequency of macro- and microscopic osteosarcomas and lymphoreticular tumours in groups of female mice (experiment A) treat ^{90}Sr ^{90}Sr + bone marrow cells and ^{90}Sr + bone marrow cells + thymic cells Dose of ^{90}Sr 25 9 kBq (0 7 μCi)/g b Dose of bone m and thymic cells 5×10⁶ The cells were given every month between one and 7 months

Group of mice	No of mice	Survival time (M±SE)	Dead without neoplasia	No of osteosarcoma bearing mice (per cent)	No of osteosarc / osteosarc bearing mouse	Survival time (M±SE)	Total No of osteosarcomas	No of lymphoreticular tumours (per cent)	Sur time (M±
I (^{90}Sr)	50	270±9 0	0	47 (94 0)	4 45	276±8 8	209	3 (6)	253
II (^{90}Sr+BM)	50	254±8 8	0	39 (78 0)	4 00	277±7 8	156	16 (32)	184
III (^{90}Sr+BM+TH)	82	287±7 9	0	79 (96 3)	4 48	291±7 8	354	4 (4 9)	300

Five also had osteosarcoma
One also had osteosarcoma
Eighteen animals were lost at the cell transfusion

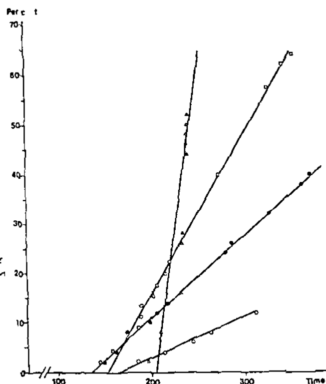
cells and their possibilities to colonize the bone marrow

During the whole experiment the mice were kept in the same room in plastic cages 10 animals in each with free supply of food and water (Astra Ewos Standard Feed for Rats and Mice)

The bone marrow cells were harvested from syngeneic female CBA mice 75 to 100 days of age After cutting both ends of 8 femurs the bone marrow was expelled by pressurized air and suspended in 0 5 ml of balanced salt solution (BSS) Then an additional 1 5 ml of BSS was added From this suspension 0 1 ml was mixed with 50 ml BSS and 0 25 ml Zaponine and counted in a Coulter Cou From each femur generally 5×10⁶ cells coul obtained From the stock suspension each m was given 0 25 ml intravenously adjusted so that volume contained 5×10⁶ bone marrow cells

The thymus was cut in small pieces homogenized in 0 5 ml of BSS and then handle the same way as described for the bone marr Thymic cells were given intravenously in dose 5×10⁶ cells suspended in 0 25 ml BSS

All mice were if possible killed in agony and before autopsy examined roentgenologically dorso ventral position The weight of the thy



Cumulative incidence of lymphoreticular tumours in relation to time (days) after treatment with ^{90}Sr (○) ^{90}Sr + bone marrow cells (●) ^{90}Sr + thymus cells (□) ^{90}Sr + bone marrow + thymus cells (△)

and spleen as well as that of the whole carcass was recorded Material was taken for histology from all cases with possible LR tumours and routinely both humeri tibiae femora pelvic bones the spine and the skull The material was fixed in Stieve s solution embedded in paraplast cut at 5 µm and routinely stained with Hematoxylin Eosin and according to the van Gieson method

Results

Experiment A The most obvious finding in series A (Table 2) was the high frequency of LR tumours in group II treated with bone marrow cells 16 of 50 animals (32%) whereas group III given both thymic and bone marrow cells had an incidence of only 4 of 82 (4 9%) The tendency to a prolonged latency time in the latter group is also notable In group I given only ^{90}Sr the incidence of LR tumours was 6 per cent and the difference between group I and II is statistically significant (χ^2=46 53 p<0 0005)

The number of osteosarcoma bearing mice and the total number of osteosarcomas are recorded in Table 2 From this it is evident that the tumour frequency was about 17 per cent lower in the bone marrow treated group (II) as compared to the other groups and further that the tumour frequency expressed as the number of osteosarcomas/osteosarcoma bearing mouse was about 10 per cent lower in group II (4 0) than in the other two groups (4 5) The survival time for the mice with osteosarcoma was practically the same in all groups in this series

Experiment B In this series (Table 3) the LR tumours were more numerous by a factor 3 4 to 5 in all the groups (VI VII VIII) treated with cells in addition to ^{90}Sr as compared to the group given ^{90}Sr alone (V) The very high frequency of LR tumours 60 per cent found in the group treated with thymic and bone marrow cells was in a sharp contrast to the low value found in the group treated in a similar way in series A (4 9%)

Even if the induction time for the LR tumours is almost the same in all groups the induction pattern differs (Figure) As indicated here the first cases of

Table 3

Frequency of macro- and microscopic osteosarcomas and lymphoreticular tumours in groups of male mice (experiment B) untreated treated with ^{90}Sr ^{90}Sr + bone marrow cells ^{90}Sr + thymic cells and ^{90}Sr + bone marrow cells + thymic cells Dose of ^{90}Sr 14 8 kBq (0 4 µCi)/g b w Dose of bone marrow and thymic cells 5×10 cells The cells were given every month with beginning at day 1 after ^{90}Sr injection

Group of mice	No of mice	Survival time (M±SE)	Dead without neoplasia	Survival time (M±SE)	No of osteosarcoma bearing mice (per cent)	Survival time (M±SE)	Total No of osteosarcomas	No of lymphoreticular tumours (per cent)	Survival time (M±SE)
IV (Control)	47	810±15 7	45	–	–	–	–	2 (4)	531
V (^{90}Sr)	49	391±17 7	1[illegible]	318±3[illegible] 1	30 (61 [illegible])	451±17 6	49	6 (12)	[illegible]49±18 4
VI (^{90}Sr+BM)	49	335±19 [illegible]	17	[illegible]98±27 8	12 (24 4)	512±20 3	20	20 (41)	260±15 7
VII (^{90}Sr+TH)	45	[illegible]46± 8 8	15	23[illegible]±17 4	[illegible] (4 4)	[illegible]25	2	29 (64)	253±10 3
VIII (^{90}Sr+BM+TH)	50	[illegible]5[illegible]±11	1[illegible]	246±[illegible]6 7	5 (10 0)	409	8	30 (60)	231± 2 4

LR tumours are observed at approximately the same day in both the groups given only one type of cells whereas in the series given both thymic and bone marrow cells the first neoplasia will appear about 50 days later The slopes of the curves also indicate that the tumour progression rate is strongly increased in all groups given cells as compared to the group given ^{90}Sr alone This is particularly obvious in the bone marrow + thymus cell group which exhibits a curve with a very steep slope and with a yield of 30 tumours within 65 days

The majority of the LR tumours obtained were as judged by histology of bone marrow origin A primary observation was the early involvement of the bone marrow in the thoracic vertebrae with or without infiltration of the surrounding tissue The soft *tissue invasion was sometimes macroscopically de*tectable as strands bilaterally along the ventral aspect of the thoracic spine or later on as large greyish nodules In the early phases the engagement of the spleen or other lymphoid tissue was insignificant In more advanced stages however when the process was more widespread the spleen and lymph nodes could be enormously enlarged but still without macroscopic signs of thymic involvement Of a total of 23 and 87 LR tumours in series A and B respectively only 2 definite thymic lymphomas were found in the former series and 4 in the latter

As a consequence of the high rate of LR tumours the mean life span was significantly reduced for the animals in group VII ($t=9\ 139$ $p<0\ 001$) and group VIII ($t=6\ 635$ $p<0\ 001$) as compared to those given ^{90}Sr alone The short life span in these groups also reflects the very low osteosarcoma incidence In the group given ^{90}Sr + bone marrow cells (VI) with an intermediate not significantly shortened survival time the frequency of osteosarcomas is higher but still much less than in the group given ^{90}Sr alone this also seems to depend largely on the high frequency of LR tumours Taking into consideration only the number of osteosarcomas/osteosarcoma bearing mouse no difference exists between the two groups (V 1 63 and VI 1 66 tumours/mouse) However it should be observed that the tumour induction time is significantly longer for group VI 512±20 3 days than group V 451±17 6 days ($t=2\ 290$ $0\ 05>p>0\ 02$)

The results obtained from the *registration* of the body and organ weights of the dead animals did not reveal any significant differences between mice treated and not treated with cells This was mainly due to the large range of the weights of the individ... mice and organs

Discussion

The distribution of ^{90}Sr in the body has been subject to a comprehensive whole body autoradiography investigation by NILSSON et coll (196?) The nuclide is after a few hours selectively concentrated in the hard tissues Initially it is mainly located in th... metaphyseal parts and along the endosteal and periosteal linings of the bones later a redistribution takes part After about 4 months the majority of the nuclide that remains in the mouse (approximately 20%) is now mainly retained in the diaphyseal re*gions whereas that retained in the metaphys...* zone has diminished considerably This redistribution of the nuclide is the main reason why the bo... marrow lesions vary in different regions of the bone at different times after its administration (NILSSON 1962 1970)

After the doses of ^{90}Sr used the lympho... haematopoietic tissues are severely damaged This is evidenced by a marked dose related depletion of all cell types in the bone marrow and as a consequence a rapid involution of the thymus and a weight increase of the spleen due to a compensatory extramedullar haematopoiesis of long duration (NILSSON 1962 1970 JÄRPLID 1973) In the bone marrow the cell depletion is most prominent after about one month when also a shift in the relative proportion between the granulocytic and erythrocytic series in favour of the former takes place whereas more and more of the erythroid cells and thrombocytes are produced in the spleen In the bone marrow the cellular depletion has a sectional distribution which is most conspicuous in the met... aphysis during the early phase after ^{90}Sr injec*tion while later on the depletion predominates in* diaphyseal areas From day 120 to 150 a regeneration of all types of cells usually starts in the bone marrow however still with a predomination for the myeloid series The bone marrow regeneration is most prominent in certain areas such as tuber s... rale tuber ischii the thoracic vertebrae and in regions around the metaphysis In late stages the *diaphyseal parts of the bone marrow is hypoplas...* and seldom regenerates at doses around the highest dose level used in the present experiments

The decreased weight of the thymus is pred...

nantly caused by a cortical depletion of lymphoid cells characterized by a diphasic pattern with a deep initial depletion followed by a sharp abortive regeneration with its peak on day 12 Thereafter the cellularity decreases until about day 30 when the weight is normalized (JARPLID 1973) Also in the spleen a strong and persistent decrease of the lymphoid tissues occurs but on account of the heavy extramedullar haematopoiesis the spleen weight will be continuously elevated and for the doses used the normalization of the weight occurs first after about 50 days (JARPLID 1973) This is mainly due to a more or less severe progressive lymphoid hypoplasia which may develop into secondary disease According to JACOBSON (1954) the haematopoietic reserve offered by the spleen is one reason why the mice could survive the initial haematologic crisis since splenectomized mice will die

In the present experiments a very high yield of LR tumours was obtained which is in complete contradiction to previous investigations with external irradiation which show that syngeneic bone marrow is very effective in protecting mice from the induction of lymphoma myeloid leukaemia and reticular cell sarcoma (KAPLAN & BROWN KAPLAN et coll COSGROVE et coll 1964) This seems to a large extent to depend on the repeated supply of cells during the course of the experiment to mice which were continuously irradiated from ^{90}Sr located in the skeleton The majority of mice were supplied with cell numbers from 25×10^6 to 100×10^6 This is a fairly large number if it is considered that a crude approximation will give a total number of about 200×10^6 bone marrow cells in a normal mouse Since the cells also were given from the very beginning of the experiment—in order to improve the general condition of the animals—most of the cells which were seeded within the bone marrow cavities were probably killed in the high dose series together with most of the bone marrow cells of the host (or severely damaged) during the first few months by the irradiation This seems to be confirmed both by the lower incidence of LR tumours in experiment A (high dose group) as compared to B and by the fact that the latency time for LR tumours (in series B) is about the same in cell treated and not cell treated ^{90}Sr groups First later when the concentration of ^{90}Sr was diminished certain sites of the bone marrow would offer an environment allowing regeneration and then first in the groups given the lower ^{90}Sr dose This means also that mice given cells will offer a largely increased number of cells at risk as compared to those given ^{90}Sr alone which seems to be the main reason for the high incidence of LR tumours

In these experiments the injected cells do not seem to colonize the spleen to the same degree as is the case after external irradiation (TILL & MCCULLOCH 1961) Virtually no spleen was found to contain colony forming units This may be associated with the fact that the radiation damage to the spleen after ^{90}Sr treatment is only minor as compared to that after high external irradiation doses leaving the spleen with an almost intact capacity of regeneration and as a consequence an insignificant need of cell import Therefore the potentially larger haematopoietic reserve of the spleen will soon be activated to overcome the acute bone marrow failure induced by ^{90}Sr A reasonable anticipation is thus that the majority of the transplanted cells will migrate to the bone marrow which also may be supported by the fact that most cases of LR tumours seem to have a bone marrow origin This is also in agreement with previous findings (NILSSON 1971) that the majority of ^{90}Sr induced LR tumours originate in the bone marrow

The large difference in the incidence of LR tumours between group II and III in experiment A could not readily be explained but may be related to the site in the body where the thymic cells are seeded Thymic cells are when injected intravenously distributed to all lymphoid organs and to the bone marrow (ZATZ & LANCE 1970) However these cells are extremely sensitive to irradiation and it would therefore be anticipated that the fraction of those homing in the bone marrow to a large extent will be exterminated and therefore not possibly constitute a large pool of potentially transformable cells This is particularly valid in the highest—less tumourogenic—dose series with the lowest incidence of LR tumours The thymic cells which are seeded in the spleen and lymph nodes or incorporated in the re circulating lymphocyte pool may survive for a considerable time (SPRENT & BASTEN 1973) thereby helping to reactivate the cell mediated immunologic defence and counteract transforming cellular clones

The most efficient ^{90}Sr dose for induction of LR tumours seems to be somewhere between 14 8 and 7 4 kBq (0 4–0 2 μCi)/g of body weight (NILSSON 1971) and it is also known that female CBA mice are more susceptible than are males (JARPLID 1974) The somewhat lower incidence in group II series A as

Table 4

Frequency of macro- and microscopic osteosarcomas and lymphoreticular tumours in groups of male mice (experiment C) trea ed ^{90}Sr and ^{90}Sr + bone marrow cells Dose of ^{90}Sr 25 9 kBq (0 7 μCi)/g b w Dose of bone marrow cells 5×10⁶ cells

Group of mice	No of mice	Survival time (M±SE)	Dead without neoplasia	Survival time (M±SE)	No of osteosarcoma bearing mice (per cent)	Survival time (M±SE)	Total No of osteosarcomas	No of lymphoreticular tumours (per cent)	Surv time (M SE
1 (^{90}Sr)	98	314± 6 8	7	246±24 8	85 (86 7)	3?8±6 1	194	8 (8)	[illegible]
2 (^{90}Sr+BM_{120})	98	312± 7 0	19	248±17 1	74 (75 5)	336±5 4	166	8 (8)	[illegible]
3 (^{90}Sr+BM_{170})	9?	339±10 4	15	361±59 3	75 (81 5)	336±5 5	190	5 (5)	[illegible]

BM_{120}/BM_{170} indicates the day bone marrow cells were given after the day of ^{90}Sr administration

Three also had osteosarcoma
Two also had osteosarcoma
Two animals lost
Eight animals killed when cells were injected

compared with group VI series B may be related to these facts i e a considerably higher frequency of LR tumours would have been expected in group A II if the dose had been 14 8 kBq (0 4 μCi)/g The highest incidence of LR tumours (64%) is observed in series B group VII which was given thymic cells alone The group given both thymic and bone marrow cells in series B had in contrast to the corresponding group in series A a high incidence of LR tumours (60%) As already mentioned 14 8 kBq (0 4 μCi)/g is a more tumourogenic dose regarding LR tumours than 25 9 kBq (0 7 μCi)/g largely because of lower cell killing effect In the lower dose group a larger number of potentially transformable cells therefore will survive making the cellular defence even if enhanced insufficient to counteract neoplastic transformation

LR tumours usually originate in the bone marrow when ^{90}Sr is given within optimum dose limits (NILSSON 1971 JARPLID 1974) This observation is in agreement with the findings in the present investigation and indicates that the cell transplantation could not modify the tumourogenic mechanism in this respect In this context it seems necessary to implicate that the increased incidence of LR tumours may be associated with a virus activation by the injected cells However no attempts have been made to unravel this possibility

On account of the high frequency of LR tumours in the cell treated groups in series B most animals died before osteosarcomas could develop However this was only partly the case in group VI where osteosarcomas developed in 12 mice as compared to 30 in group V In both these groups number of osteosarcomas/osteosarcoma bea mouse was the same 1 6 The significantly lor survival time of the bone tumour bearing mice group VI may perhaps be considered as an impli tion however vague of a reinforcement of the h antitumour defence The findings in series A that number of bone tumours per mouse was slightly l in group II than in group I (Table 2) may also taken as a weak indication in this direction supported by the results in group III howev Since these observations were difficult to evalu and partly were contradictory a new experimen series C—was inserted in which modifications w undertaken as regards the most convenient time bone marrow transplantation and number of c injected Three groups each of 100 CBA male m were given 25 9 kBq (0 7 μCi) ^{90}Sr/g b w intrap toneally One group (1) was given only ^{90}Sr and t were given 5×10⁶ bone marrow cells in a single travenous injection on day 120 (2) and 170 (3) spectively after the nuclide administration The sults of these experiments are recorded in Table The time 120 days was chosen because it seems offer one of the earliest opportunities for the tra planted cells to successfully repopulate the b marrow Day 170 was selected also with the poss aim to interfere with the earliest preneoplastic p liferations which with the doses administered do cur at this time (NILSSON 1970) with the exceptio of the rare occasions of earlier appearance

In the inserted experiment C a few observa were made which may help to explain the results

the original investigation Thus the frequency of LR tumours was not enhanced in the cell treated groups because in contrast to the earlier experiments precautions *were taken not to provoke* tumourogenesis by giving the transfusion as a single dose long enough after ^{90}Sr to assure a less risky environment to the transplanted cells Consequently the number of cells at risk was held within limits A slight indication of a beneficial effect of cell treatment may also appear from an increased life span for the animals given cells at day 170 (versus ^{90}Sr only $t=2.0113$ $0.02<p<0.05$) Also animals dying without neoplasia show a tendency to have a longer life span in group 3 than in group 1 (however not significant $t=1.788$ $p=0.10$)

From the results of experiment A and B the anticipations arose that cell injections may have a stimulatory effect on the antitumour defence This conclusion is very uncertain and could not be unambiguously answered by the inserted experiment nor is it supported by COSGROVE et coll or COVELLI et coll who reported that neoplasms other than LR tumours were practically unaffected by bone marrow transplantation A χ^2 analysis of the tumour material in Table 4 reveals an almost significantly lower tumour frequency if group 1 is compared with group 2 ($\chi^2=5.272$ $0.010<p<0.025$) whereas group 1 versus 3 does not fulfil the statistical standards of significance ($\chi^2=2.712$ $p=0.10$)

Whatever the truth may be as regards the possibility to reactivate the haematopoiesis the specific and unspecific defence system as well as the general condition of heavily contaminated animals it seems necessary to point out the enhancing risk of LR neoplasia when transplanting lympho-haematopoietic cells to individuals contaminated with bone seeking nuclides

It also seems to be of a biologic interest to investigate more exactly the optimum injection time and cell doses which can be given without exacerbating the induction of LR tumours but utilize the beneficial effect which the present experiments indicate In this context it also seems to be of interest to analyse if and in which way bone marrow transplants may increase the population of the very ^{90}Sr sensitive natural killer cells (KIESSLING & WIGZELL 1979) in animals irradiated with this nuclide at different times after ^{90}Sr injections and to what an extent these cells during more optimum conditions may be able to counteract tumour progression

SUMMARY

Mice irradiated with ^{90}Sr in doses of 14 8 and 25 9 kBq/g body weight were given bone marrow or thymic cells *intravenously at monthly intervals or in single doses at* 120 or 170 days after injection of ^{90}Sr At the low dose level a high incidence of lymphoreticular tumours in all cell treated groups occurred as compared with animals irradiated with ^{90}Sr only At the high dose level only the bone marrow transplanted group contracted a high incidence of lymphoreticular neoplasia Furthermore a somewhat decreased osteosarcoma incidence in the cell transplanted animals appeared to be indicated However the results obtained are inconsistent and difficult to evaluate Therefore it seems necessary to repeat the experiments if more precise conclusions should be possible to draw

ACKNOWLEDGEMENT

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Table 4

Frequency of macro- and microscopic osteosarcomas and lymphoreticular tumours in groups of male mice (experiment C) treated with ^{90}Sr and ^{90}Sr + bone marrow cells Dose of ^{90}Sr 25 9 kBq (0 7 μCi)/g b w Dose of bone marrow cells 5×10⁶ cells

Group of mice	No of mice	Survival time (M±SE)	Dead without neoplasia	Survival time (M±SE)	No of osteosarcoma bearing mice (per cent)	Survival time (M±SE)	Total No of osteosarcomas	No of lymphoreticular tumours (per cent)	Survival time (M±SE)
1 (^{90}Sr)	98 *	314± 6 8	7	246±24 8	85 (86 7)	378±6 1	194	8 (8)	0 [illegible]
2 (^{90}Sr+BM_{120})	98 *	312± 7 0	19	248±17 1	74 (75 5)	336±5 4	166	8 (8)	236+[illegible]
3 (^{90}Sr+BM_{170})	92	339±10 4	15	361±59 3	75 (81 5)	336±5 5	190	5 (5)	67 [illegible]

BM_{120}/BM_{170} indicates the day bone marrow cells were given after the day of ^{90}Sr administration

Three also had osteosarcoma
Two also had osteosarcoma
Two animals lost
Eight animals killed when cells were injected

compared with group VI series B may be related to these facts i e a considerably higher frequency of LR tumours would have been expected in group A II if the dose had been 14 8 kBq (0 4 μCi)/g The highest incidence of LR tumours (64%) is observed in series B group VII which was given thymic cells alone The group given both thymic and bone marrow cells in series B had in contrast to the corresponding group in series A a high incidence of LR tumours (60%) As already mentioned 14 8 kBq (0 4 μCi)/g is a more tumourogenic dose regarding LR tumours than 25 9 kBq (0 7 μCi)/g largely because of lower cell killing effect In the lower dose group a larger number of potentially transformable cells therefore will survive making the cellular defence even if enhanced insufficient to counteract neoplastic transformation

LR tumours usually originate in the bone marrow when ^{90}Sr is given within optimum dose limits (NILSSON 1971 JARPLID 1974) This observation is in agreement with the findings in the present investigation and indicates that the cell transplantation could not modify the tumourogenic mechanism in this respect In this context it seems necessary to implicate that the increased incidence of LR tumours may be associated with a virus activation by the injected cells However no attempts have been made to unravel this possibility

On account of the high frequency of LR tumours in the cell treated groups in series B most animals died before osteosarcomas could develop However this was only partly the case in group VI where osteosarcomas developed in 12 mice as compared to 30 in group V In both these groups the number of osteosarcomas/osteosarcoma bearing mouse was the same 1 6 The significantly longer survival time of the bone tumour bearing mice in group VI may perhaps be considered as an implication however vague of a reinforcement of the host antitumour defence The findings in series A that the number of bone tumours per mouse was slightly less in group II than in group I (Table 2) may also be taken as a weak indication in this direction not supported by the results in group III however Since these observations were difficult to evaluate and partly were contradictory a new experimental series C—was inserted in which modifications were undertaken as regards the most convenient time for bone marrow transplantation and number of cells injected Three groups each of 100 CBA male mice were given 25 9 kBq (0 7 μCi) ^{90}Sr/g b w intraperitoneally One group (1) was given only ^{90}Sr and two were given 5×10⁶ bone marrow cells in a single intravenous injection on day 120 (2) and 170 (3) respectively after the nuclide administration The results of these experiments are recorded in Table 4 The time 120 days was chosen because it seems to offer one of the earliest opportunities for the transplanted cells to successfully repopulate the bone marrow Day 170 was selected also with the possible aim to interfere with the earliest preneoplastic proliferations which with the doses administered do occur at this time (NILSSON 1970) with the exception of the rare occasions of earlier appearance

In the inserted experiment C a few observations were made which may help to explain the results

FROM KING FAISAL SPECIALIST HOSPITAL AND RESEARCH CENTRE DEPARTMENT OF MEDICAL PHYSICS
RIYADH SAUDI ARABIA

SIMPLE EXPERIMENTALLY DERIVED ALGORITHM FOR COMPUTER CALCULATED DOSE RATES ASSOCIATED WITH ^{137}Cs GYNECOLOGIC INSERTIONS

D E WREDE and H DAWALIBI

POWERS et coll compared the MIR (Mallinckrodt Institute of Radiology) program with three well known programs in 1966 The three programs were by SHALEK & STOVALL (1962 1968) ADAMS et coll (1964) and ADAMS & MEURK (1964) Radium was used as the nuclide The second and third programs are for intracavitary treatment and the first program for interstitial treatment only

Their programs were prompted by the fact that a difference in dose of the order of 20 per cent at a particular point occurred An interesting point was illustrated i e the distance to be moved for normalization of the dose for the several programs is only a few millimeters Thus because of the steep gradients tha occur in intracavitary (and even more in interstitial) implants large per cent differences may occur for either a calculated or measured dose rate at a specified point This led to the conclusion that accuracy is best expressed in terms of distances to be moved in order to obtain agreement between different programs rather than trying to compare measured or calculated dose rates

Another interesting observation they made was that the maxim at distances from a source equal to or greater than two times the length of the source the dose variation is nearly approximated by an inverse square function is nearly true and can be used as a rapid approximation of dose without the necessity of performing explicit calculations in the estimation of doses from intracavitary applicators

Finally they suggested that an ideal program would have a simple rapid and accurate input a concise and accurate computation method using reasonable factors and logic and a clearly meaningful output This latter statement summarizes the basis for this report

Conventional computational methods

One of the well known methods for intracavitary dose calculations is the SHALEK & STOVALL (1968) interval method for determining dose rate to a point P from a linear ^{137}Cs source and is based upon dividing the active length into N point sources and summing the contributions from each The basic formula for one source is

$$(D_p)_j = \frac{\Gamma_{Cs} A f}{N} \sum_{1}^{N} \left[\frac{e^{-\mu t_i}}{d_i}\right] \times T(d_i) \qquad (1)$$

where

$(D_p)_j$ = the dose rate at the point p from the jth source
Γ_{Cs} = the exposure rate constant for ^{137}Cs
f = rad/roentgen conversion factor for muscle tissue
A = the activity of the source
μ = the linear attenuation coefficient of the wall materials for ^{137}Cs photons
d = the distance from the point P to the center of the ith point source of a source
t_i = the path length within the filter for a ray from the ith point source

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$T(d_i)$ = a tissue correction factor compensating for absorption and scatter

N = number of point sources assumed per actual active length of a single source

This formula would be applied to all sources and the results all summed for point P

BREITMAN (1974) goes into some detail expanding upon the definition and empirical fitting of the terms mentioned and derives by computer calculation a table of dose rates for a variety of ^{137}Cs tube geometries Active lengths are for 1 35 cm 1 5 cm 3 0 cm and 4 5 cm and for a variety of filtrations and active diameters as well

Using tables published by SHALEK & STOVALL (1968) as a basis for comparison between radium sources and cesium sources having the same construction and equivalent radium content the dose distributions were found to be similar with the exception of the heavily filtered ends where 9 per cent difference occurred due in part to the complex spectrum of radium

When BREITMAN s result is compared with KRISHNASWAMY s theoretical data (1972) for stainless steel encapsulation agreement is found only on the transverse axis KRISHNASWAMY s tables were derived from MIRD (Medical Internal Radiation Dose Committee of the Society of Nuclear Medicine) data according to BERGER (1968) The two methods agreed to within one per cent at most points when the computer method was used to generate a table for a steel filtered cesium needle using a linear energy absorption coefficient of 0 221 cm $^{-1}$ based on HUBBELL s data (1960) This procedure confirmed (1) the equivalence of the two approaches to calculating dose rates and the conclusion that the dose distribution of platinum filtered ^{137}Cs sources is more closely related to that of platinum filtered ^{226}Ra sources of the same size than to stainless steel filtered ^{137}Cs sources Actually the first part of this conclusion was determined much earlier by HORWITZ et coll (1964) and others including the first author An explicit simple power expression for dose rate was derived from a ^{137}Cs point source in an infinite unit density medium based upon the Monte Carlo derived data (BERGER) after observing that a plot of log of specific absorbed fraction versus the log of distance from the point source formed a straight line

A least squares fit gave the expression

$$D=\frac{k_1}{d^k}\times A \qquad (2)$$

where D=dose rate (Gy/h) in an infinite unit density medium at a point d cm from the point source of ^{137}Cs of A activity (in Bq) and k_1 and k are constants obtained from a least squares fit

Computer algorithm

Using the theoretically derived expression for dose rate from a point source a simple program can be written in which a simple do loop is utilized to break the source up into any number of pieces such that each piece i of linear source j can be treated as a point source The total dose rate to a given point of interest p is thus given by a simple numerical integration i e

$$D_i=\sum_{j=1}^{M}\sum_{i=1}^{N}\frac{k_1 A_{ij}}{d_{ij}^k} \qquad (3)$$

where

$$d_{ij}=[(X_{ij}-X_p)^2+(Y_{ij}-Y_p)^2+(Z_{ij}-Z_p)^2]^{\frac{1}{2}} \qquad (4)$$

X_{ij} Y_{ij} Z_{ij} = the coordinates of the ith point source of the jth source

X_p Y_p Z_p = the coordinates of the point of interest

A_{ij} = the activity of the ith point source of the jth source

M = the number of linear sources

N = the number of point sources specified per each real source active length

D_p = the total dose rate at point p from all sources

Program features such as the provision to go back and easily correct coordinates that were typed incorrectly into the terminal the ability to modify source strengths and thus optimize point A-rectal (or bladder) dose rate differentials use of the conversational mode the ability to print out dose rates in specified planes of interest (one centimeter actual spacings) etc provide a useful versatile program Basically the only input data required are (1) The x y z coordinates at the end of each active length (2) the activity in mCi of each source (3) the lateral and a p radiographic magnification factor (4) the source decay factor and (5) spatial coordinates of points of

interest Data can be extracted from a set of orthogonal films inputted into a conventional teletype terminal and outputted all in less than half an hour The same program can of course also be implemented on a small dedicated type computer and the coordinates inputted using a digitizer

Comparison between theoretical dose rates

A comparison of theoretical dose rates for a wide selection of clinical points of interest between the present data and those of KRISHNASWAMY agreed within one per cent on the perpendicular bisector for a single 2 cm source of active length 1 2 cm This is of no great surprise since both were derived from the same basic MIRD data according to BERGER Slight differences of the order of 3 per cent occurred at points not lying on the transverse axis due to the fact that the present model did not include the effects of oblique filtration although it did inherently contain the effect of 0 5 mm of platinum on the transverse axis In an attempt to retain the same simple analytical algorithm experimental data were obtained from a Rando Alderson phantom and the constants k_1 and k_2 were adjusted in order to force agreement between the computer printout and the experimental data

General experimental procedure

A ^{137}Cs source (OL=2 0 cm AL~1 2 cm filtration=0 5 mm platinum) was placed at approximately the external os position in the Rando Alderson phantom LiF (TLD 700 powder) detectors were placed in the same place of the phantom i e on the perpendicular bisector of the source and in places above and below the source Each slab was 2 5 cm thick but powder only occupied the central 1 2 cm portion of each slab corresponding to the active length of the source Irradiations were carried out over both a 17 hour and an 18 5 hour time interval and averages of readings were recorded The powder was read out on a Harshaw TLD Unit and readings were used to determine doses and therefore dose rates using a calibration curve based upon the known outputs of a ^{60}Co teletherapy unit Readings were plotted against length of the powder and the peak at the center point was taken as the correct reading for determining the dose A contrast medium (Hypaque) was next placed into the teflon capsules which had been used to hold the LiF powder and the capsules repositioned into the phantom at the same positions which the LiF capsules had occupied A p and lateral films were exposed and coordinates corresponding to the ends of the active source and centers of the Hypaque filled capsules were carefully measured from the film (Actually all coordinates were known by geometry but the radiographic procedure allowed for a better simulation corresponding to the routine clinical practice) The existing computer program was used to calculate dose rates at the measured points and these dose rates were compared with the directly measured dose rates

Comparison of theoretical and experimental dose rates

Differences varied from 4 to 10 per cent between the experimental and computer derived data By adjusting k_1 and k_2 differences were minimized to within 2 per cent except at extreme oblique positions The differences within a solid angle produced by ±45 relative to the transverse axis at the center of the source were less than 2 per cent This is the same magnitude of difference when calculating with precisely known spatial coordinates as compared with coordinates obtained from a set of orthogonal films

After adjusting k_1 and k_2 differences of dose rate along the transverse axis for a single source varied systematically from the data of KRISHNASWAMY the difference being 10 per cent at a distance of 7 cm At a distance of 2 cm the difference was 5 per cent In all cases the experimental (or computer generated value after adjustment of k_1 and k_2) dose rates were always lower This is intuitively expected since the theoretical model is based on an infinite medium and the experimental data are based upon a finite medium (the Rando Alderson phantom) As one proceeds further from the source less scatter component would be expected in the finite medium thus reducing the dose rate from that which would occur in an infinite medium

Conclusion

POWERS et coll have suggested that an ideal program would have a simple rapid and accurate input and a concise and accurate computation method using reasonable factors and logic as well as a clearly meaningful output The authors feel as if the

present algorithm provides the basis for such a program An analytical expression in lieu of table lookup allows conciseness reduction of required computer memory and the accuracy of the computation is assured by the agreement (±2%) with experimentally derived data using a female Rando Alderson phantom The input parameters are simple and can be quickly ascertained Much of the accuracy depends on the individual Spatial coordinates can be obtained directly from a set of orthogonal films by hand or can be inputted directly using a transducer The output is meaningful and useful in afterloading techniques if one allows for the printout of dose rates from individual sources as well as the summed dose rates from all sources for any point of interest This is useful since the source strengths can easily be modified within the computer until more desirable differential dose rates are obtained The results are also interesting in that not only is energy build up and attenuation accounted for in the unit density medium but finiteness of the medium appears to have an effect reducing the dose rate with distance from the source as compared with theoretically expected values In practice this is of no great consequence though since points of clinical interest such as points A B C rectum bladder etc lie relatively close to the sources

SUMMARY

A simple mathematical algorithm is derived from experimental data for dose rates from ^{137}Cs sources in a finite tissue equivalent medium corresponding to the female pelvis An analytical expression for a point source of ^{137}Cs along with a simple numerical integration routine allows for rapid as well as accurate dose rate calculations at points of interest for gynecologic insertions When compared with theoretical models assuming an infinite unit density medium the measured dose rates are found to be systematically lower at distances away from a single source 5 per cent at 2 cm and 10 per cent at 7 cm along the transverse axis Allowance in the program for print out of dose rates from individual sources to a given point and the feature of source strength modification allows for optimization in terms of increasing the difference in dose rate between reference treatment points and sensitive structures such as the bladder rectum and colon

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Copies of the computer program written in Fortran are available from the authors upon request

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FROM THE CANCER RESEARCH AND TREATMENT CENTER UNIVERSITY OF NEW MEXICO ALBUQUERQUE NEW MEXICO 87131 USA

COMPUTATION OF DOSE DISTRIBUTIONS FOR RADIOACTIVE SEED IMPLANTS

I I ROSEN R G LANE and C A KELSEY

Interstitial and intracavitary implants with radioactive seeds permit irradiation of localized tumors with higher doses to tumor and lower doses to surrounding normal tissue than are possible with external beams Temporary implants are typically used for carcinoma of the cervix vagina rectum oral cavity and oropharynx Permanent implants are typically used for malignancies in such sites as the prostate lung bladder and lymph nodes Some review articles on implantation technique dosimetry and clinical results include ANDERSON (1975) SYED & FEDER (1977) HILARIS (1968 1976) HILARIS et coll (1974 1975 1976) and KIM & HILARIS (1975)

Tumor and normal tissue doses from an implant are sensitive to source geometry because of the localized distribution of dose from each seed Although some of these implants can be approximated by models each implant is unique and in general requires a dose calculation based on the actual source configuration In principle dose distributions of an implant could be calculated by hand from the distribution of the individual seeds however in practice hand calculations for doses at more than a few points are impractical STOVALL & SHALEK (1972) have summarized the computer methods used for all types of interstitial and intracavitary dosimetry

Material and Methods

An interactive computer program for computation of dose distributions from radioactive seed implants is described The program can handle up to 300 ^{125}I or ^{192}Ir seeds Seed coordinates can be entered using either the keyboard or a sonic digitizer from either orthogonal or stereo films The program produces a data file containing a dose matrix This output data file can be combined with the output of other treatment planning programs to provide total treatment plans for combined modalities Separate programs display and plot the isodose distributions The last case run is saved in a temporary file therefore until a new case is entered the last case can be recalled easily for additional plans or for modification and new plans if the implant was changed The program is run on a PDP 11/40 computer from a Tektronix graphics terminal A Versatec printer/plotter is used for hardcopy alphanumeric output and the isodose distributions are plotted on a Houston Instruments pen plotter

The program manipulates seed information in units of ribbons although this term is not applicable to ^{125}I sources Each ribbon is defined by the user and may have from 1 to 300 seeds therefore a ribbon may consist of a single seed an actual ribbon (^{192}Ir) an entire plane or even the entire implant All seeds in a given ribbon have the same activity however the activity of the seeds in different ribbons may be different The source coordinates are entered in groups by ribbon

When the sonic digitizer is used for computer input the origins and axes of the films need be entered only once They can be re-entered if the films have

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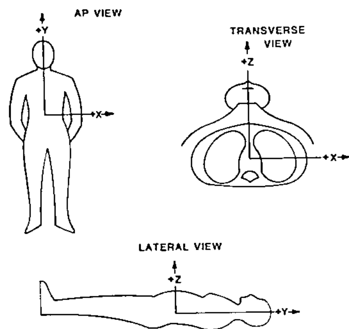


Fig 1 Reference coordinate system The standard planes of calculation Arbitrary planes of calculation are specified by the coordinates of three points relative to this coordinate frame

Seed

Te t case 1 00-00-00
Ir 0 10 to 0 30 mg
21 seeds in 3 ribbons
Seed coo dinates from stereo f lms
Target film-d stance = 120 cm
Displacement angle = 8 degrees
1 isodose plan

EN	Enter a new case (change all)
SA	Save this case permanently
CF	Change film factors
DR	Delete a ribbon—type number
AR	Add a ribbon
CA	Change act v ty of a ribbon—type number
LS	List the seeds—type ribbon number (0 all)
PS	Print the seeds on the Versatec
CS	Change a seed—type number
LP	List the isodose plans
DP	Delete a plan—type number
AP	Add a plan
CO	Cont nue with the calculation
—	End the program

Option

Fig 2 Main display of the program A summary of the
with a list of command options Some of the options gi
detailed information about the implant

been moved between digitizing ribbons The number of seeds in a ribbon is entered at the keyboard and the number entered via the digitizer is checked against the number expected

For ^{192}Ir seeds the activity of each seed is entered in equivalent mg of radium The program then computes a dose rate distribution in Gy/h The dose rate at 1 cm in tissue is calculated from the gamma factor and f factor to be 0 0792 Gy/mg h (7 92 rad) Doses at all other points are calculated using the inverse square law

The activity of ^{125}I seeds is entered in MBq The dose rate as a function of distance in Gy(mCi)/h is tabulated from the graph given by ANDERSON The dose rates at all points are computed from the table using linear interpolation for intermediate values Then because these implants are permanent the dose rate at each point is multiplied by the mean life of ^{125}I (2073 6 h) to give the dose at that point resulting from the total decay of the seeds

A reference coordinate system for all seed positions is used based on standard antero posterior lateral and transverse views (Fig 1) These standard isodose plans including displacements from the origin may be requested directly Arbitrary planes of calculation are specified by three points whose coordinates are given using the patient coor dinate frame The location of the coordinate syste origin is arbitrary However the y axis and the position of the origin must be the same on both fil of an orthogonal or stereo pair

The implant films are taken on a Picker isocentr simulator For orthogonal films the isocenter placed at the center of the implant Antero-posteri and lateral films are exposed sequentially by rotati the unit The magnifications are computed from th source to-axis and source to film distances For ob taining stereo films the patient is placed on a su port frame with a space in it for the film cassett The film is located on the table top directly belo the patient The patient is raised so that the is center of the machine is set at the film The cer of the field is therefore the same point in space f both stereo films and is used as the coordi origin when digitizing the films Since the fie center appears on the films no additional mark is needed The same displacement angle from th vertical about the y axis is used for both ster films Typically a source to-film distance of 120 c and a displacement angle of 8° are used

Standard a p lateral and transverse isod plans can be requested by a single command T

of the plan may be entered or the computer e the average value of all the actual seed iates Displacements from the origin can be ed to give sectional views The grid limits the area over which the doses are to be calcu They may be entered or the computer will id limits that are at least 2 cm greater than ost extreme source coordinates in each direc ounded off to the nearest centimeter

both orthogonal and stereo films the y-coor of a source is independently computed from ilm An uncertainty in the value Δy is then ited This uncertainty is displayed or printed ition to the source coordinates This delta is useful in determining if the patient moved ntially between the films or if a digitizing error ed The y value used for computing isodose utions is the average of the two independent

main display of the program appears in Fig 2 etter mnemonics are used for specifying com options Where a seed ribbon or plan number uired that number may be entered with the and mnemonic For example isodose plan er 1 can be deleted by typing DP1 If the er is omitted the computer will issue an ap iate prompt The available commands are iented upon as follows

Enter all new information If an error is en countered in retrieving the last case this op tion is automatically invoked

Save this case in a permanent file The perma nent file intended for statistical or other re trospective analysis will be named according to the patient s name and the isotope used

Change the film factors The factors changed depend on whether the films are orthogonal or stereo This option will cause a recomputation of the actual coordinates and the Δy values of the sources

Delete a ribbon

Add a ribbon

Change the activity of a ribbon

List the seed data on the terminal The data are listed by ribbon with the ribbon activity shown Such data include the film coordinates actual coordinates and Δy for e seed

Print the seed data on the printe The same information as that in the LS command is printed

CS Change the coordinates of a single seed

LP List the isodose plans to be calculated on the terminal The information presented is the plan description the coordinates of the three points defining the plane and the calculation grid limits

DP Delete an isodose plan

AP Add an isodose plan Up to seven isodose plans can be specified Standard antero pos terior lateral and transverse views can be re quested by specifying the option AP LA and TR respectively Arbitrary views can be ob tained by entering the coordinates of three points defining the plane When standard plans are specified the three points defining the plane are calculated by the computer and listed with the LP command

CO Continue on to the calculation of the isodose plans

← (Carriage return) Terminate the program without calculating any isodose distributions All data currently in the program are saved until a new case is entered

Conclusions

This treatment planning program is being used routinely for $^{1\ 5}$I and ^{19}Ir implants It has been found to be easy to use For every implant both orthogonal and stereo films are exposed Whichever pair is easier to evaluate is used for computer input The orthogonal pair is used for adding anatomy to the isodose distributions The automatic choices for isodose plans make selection easy and reduce treat ment planning time both by reducing input time and by reducing errors resulting from incorrect specifi cation of the desired plan The program is easily extendable to other seeds such as radon and gold as the need arises

SUMMARY

An interactive treatment planning program for the com putation of dose distributions for ^{125}I and ^{192}Ir seed im plants of up to 300 sources is described The seed coordi nates are entered using either the keyboard or a sonic digitizer from either orthogonal or stereo films The pro gram produces a dose matrix which can be displayed and plotted directly or combined with the output of other treatment planning programs to provide composite plans for treatments using multiple modalities Seeds of different

activities can be combined within a single plan The program uses two-letter mnemonics for specifying the available options among which are commands for choosing standard antero posterior lateral and transverse plans

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EFFECTS OF WASHING ON PHYTOHEMAGGLUTININ RESPONSIVENESS OF LYMPHOCYTES FROM IRRADIATED PATIENTS

S MATSUBARA J HORIUCHI H SHIBUYA and M S SASAKI

Immunity against malignancy is believed to be redominantly a cellular mechanism involving imor cell destruction by lymphocytes of thymic ngin (WATERS 1978) The responsiveness of lym hocytes to phytohemagglutinin (PHA) stimulation hich is related to delayed hypersensitivity de reases with ionizing radiation (BARAL & BLOM REN 1976 RICKINSON & ILBERY 1971 STEWART & 'EREZ 1976) This phenomenon occurs in lympho ytes irradiated in vitro as well as in peripheral ymphocytes obtained from patients being irradiated or malignant disease (BLOMGREN et coll 1974 ɹLAS & WASSERMAN 1974 HOPPE et coll 1977 IL ɪERY et coll 1971 ORDER 1977) In addition several authors have reported generally lowered PHA reac ivity in patients with malignant tumors before the beginning of any kind of therapy (MANNICK et coll 977 MCLAUGHLIN & BROOKS 1974 VETTO et coll 975)

Irradiated peripheral lymphocytes have been washed for analysing the PHA responsiveness and he results are now reported MANNICK et coll observed that non PHA responding lymphocytes of patients with malignant tumors after washing with a ulture medium and incubation with normal serum xhibited an improved level of transformation Therefore the frequency of chromosomal aberra ions afforded by washing was also recorded

Materials and Methods

In 60 patients who received radiation therapy for malignant tumors by fractionated doses of ^{60}Co γ rays the changes in peripheral lymphocyte count were recorded

These patients had a variety of malignant tumors including carcinomas of the head neck lung and esophagus testicular tumors and malignant lym phomas The patients (22 females 38 males) varied in age from 9 to 81 years with a mean age of 59 The tumor dose ranged from 30 Gy per 3 weeks to 75 Gy per 9 weeks Absolute lymphocyte counts were de termined by multiplying the peripheral white blood cell count by the percentage lymphocyte in the dif ferential and were displayed in relative value against the initial counts In 16 of the 60 cases chromosome analysis of the peripheral lymphocytes was per formed at the completion of the radiation therapy

This analysis was repeated one to three times in the following 9 weeks in order to evaluate the fluc tuation of chromosome aberration frequencies

In 4 other irradiated cases not included in the series of 60 cases (Table 1) in which all known carcinoma had been removed operatively before ir radiation PHA response and chromosome aberra tion frequencies were recorded using both direct and wash methods as described in the following These patients were selected to exclude the possible effect of existing carcinoma on the inhibition of PHA response

The blood used for the in vitro experiments was obtained from healthy young donors An aliquot of the blood was irradiated in plastic tubes at room temperature by tele cobalt unit to a total dose of

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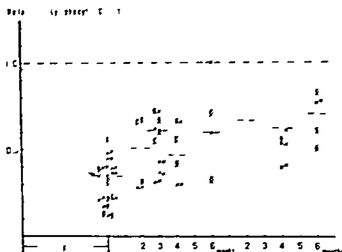


Fig 1 Lymphocyte counts after irradiation in relation to pre irradiation value

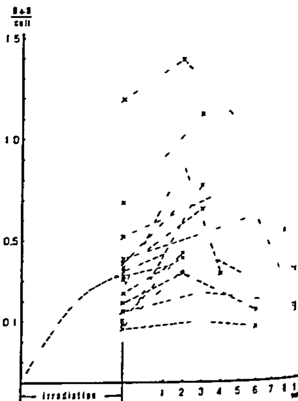


Fig 2 Frequency of chromosome aberrations in peripheral blood lymphocytes after end of irradiation

either 4 Gy or 6 Gy using a dose rate of 0 82 Gy/min For the determination of PHA responsiveness and chromosome aberration frequencies lymphocyte containing blood was cultured in the medium RPMI 1640 supplemented with 20 per cent of fetal calf serum (Gibco) and 3 per cent of PHA M (Gibco) Colcemid (0 5 μg/ml) and thymidine (5×10^{-5} M) were also added to all cultures at 24 hours of incubation

The cultures established from the irradiated cases as well as those from the healthy donors with without irradiation were harvested at varying times of culture The effect of washing with culture medium was recorded by comparing the results obtained from direct cultures with those from cells washed and suspending in culture medium for 18 hours before incubation

Chromosome preparations were made according

Table 1

Summary of clinical data and sampling time

Case No	Blood sampling	Interval between end of therapy and examination	Age and sex	Disease	Radiation dose
1	a	Immediately after the therapy	27 M	Testicular tumor	30 Gy in opposing fields
2	b	24 hours	42 M	Testicular tumor	33 Gy in opposing fields
3	c	4 days	45 F	Carcinoma of uterine cervix	47 Gy in opposing fields
4	d	31 days	45 M	Testicular tumor	45 Gy in opposing fields
	e	6 months	45 M	Testicular tumor	45 Gy in opposing fields

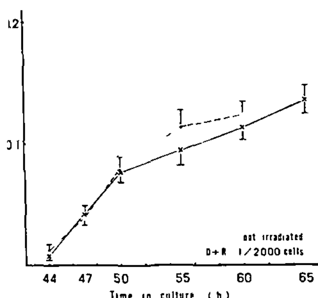


Fig 3 Mitotic index in cultures of non irradiated lymphocytes as a function of time in culture Direct culture (——) Culture of lymphocytes after washing (– – –)

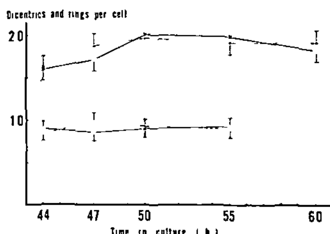


Fig 5 Frequency of chromosome aberrations in cultures of lymphocytes irradiated in vitro after 6 Gy (upper curves) and 4 Gy (lower curves) Direct culture (——) Culture after washing (– – –)

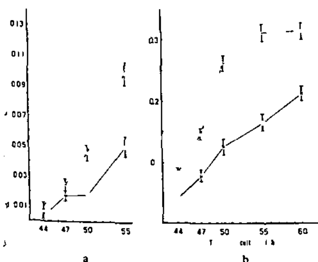


Fig 4 Mitotic index in cultures of lymphocytes irradiated in vitro after a) 4 Gy and b) 6 Gy Direct culture (——) Culture after washing (– – –)

to the standard procedure which included hypotonic treatment with 0 9 per cent sodium citrate fixation in a methanol acetic acid (3 1) and flame drying The slides were stained with Giemsa and analysed for mitotic indices and chromosome aberration yields *One hundred mitoses were then examined* for the presence of dicentrics rings abnormal monocentrics and acentric fragments In 2 cases with poor PHA response only 62 and 72 mitoses were analysed In non irradiated cases 1000 mitoses were examined in the preparations harvested at 55 hours of incubation and made by direct and wash methods respectively At other incubation times 200 mitoses were analysed in each series For the evaluation of PHA responsiveness mitotic index was determined on the basis of 2 500 mononuclear cells

Results

The changes in the peripheral lymphocyte count *following the radiation therapy appear in Fig 1 The* number of lymphocytes at the end of irradiation usually the minimum value was approximately 35 per cent of the initial count though the value varied possibly due to differences in radiation dose and field The decrease in lymphocyte count was followed by a slight recovery usually occurring within about 3 weeks of the end of radiation therapy Further improvement was found to be slight

The chromosome analyses made in 16 of the 60 patients revealed a temporary rise in aberration frequency 2 to 3 weeks after the end of irradiation and a gradual decrease thereafter (Fig 2) The coincidence *of a slight recovery of* peripheral lymphocyte count with the rise in chromosome aberration frequency may be a reflection of a temporary replenishment of blood lymphocytes by previously irradiated lymphocytes *stored in the extravascular* system The radiation induced delay in mitotic pro-

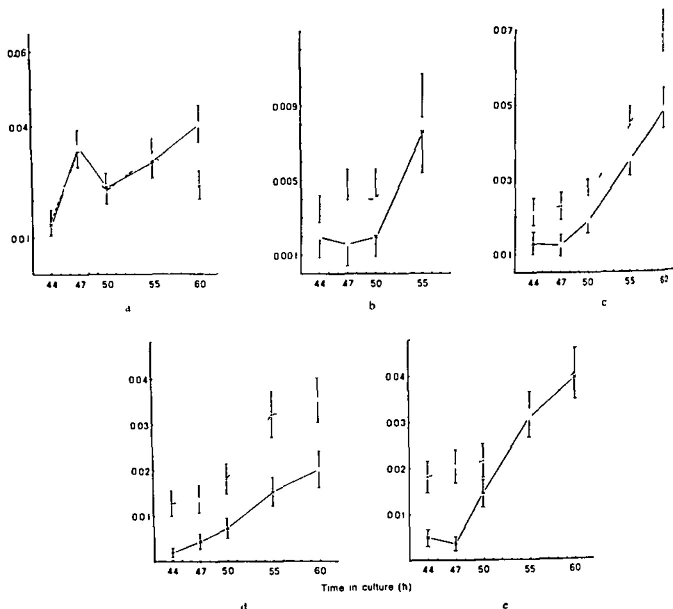


Fig 6 Mitotic index in cultures of lymphocytes from patients receiving irradiation a) Immediately after completion of therapy b) after 24 hours c) after 4 days d) after 31 days and e) after … months Direct culture (——) Culture after washing (- -)

gression and hyporesponsiveness to PHA may also influence the chromosomal aberration yields

The PHA reactivity of non irradiated peripheral lymphocytes was not significantly different between direct and wash methods as determined by the change in mitotic index against culture time (Fig 3) However lymphocytes irradiated with 4 Gy in vitro showed higher mitotic indices in washed condition than in direct cultures (Fig 4a) The increase was even more apparent in lymphocytes irradiated with 6 Gy in vitro (Fig 4b)

Chromosome analysis in non irradiated lymphocytes using the direct method and harvested at 55 hours showed only one dicentric chromosome and no dicentric was found in culture made after washing The yields of dicentrics and rings in lymphocytes irradiated in vitro with 4 or 6 Gy showed no change in the frequency of chromosome aberrations with or without washing (Table 2 Fig 5)

As for the PHA responsiveness of lymphocytes from patients receiving radiation therapy the peripheral lymphocytes taken immediately after the completion of irradiation showed no improvement in PHA responsiveness by washing (Fig 6)

An increase in mitotic indices caused by washing became evident in the lymphocytes obtained 4 or more days after the end of radiation therapy The increase in mitotic index associated with washing

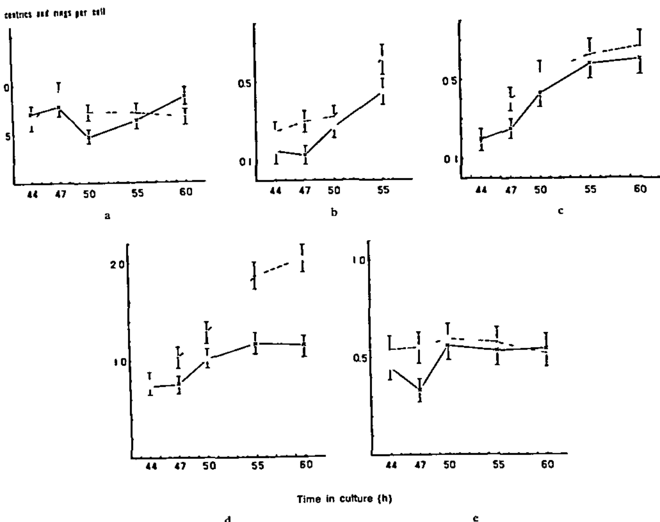


Fig 7 Frequency of chromosome aberrations in cultures of lymphocytes from patients receiving irradiation a) Immediately after completion of irradiation b) after 24 hours c) after 4 days d) after 31 days and e) after 6 months Direct culture (——) Culture after washing (---)

was most remarkable in lymphocytes obtained 31 days after the end of irradiation (Fig 6) However the lymphocytes taken from the patients 6 months after the end of radiation therapy showed only slight hyporeactivity in unwashed condition

The changes in chromosome aberration yields against the time in culture are presented in Fig 7 The aberration frequency increased with the increase in culture time particularly in cultures with improvement of the mitotic index by washing (Table 3 Fig 7) Washing of lymphocytes showed a further increase in chromosomal aberration yields which were still culture time dependent The frequency of dicentrics and rings among the cells with unstable chromosome aberrations as expressed by Qdr values also increased with the prolongation of incubation time (Table 3)

Discussion

The peripheral lymphocyte is one of the cells most sensitive to radiation Its number decreases rapidly with increase in radiation dose (BARAL et coll 1977 BENJAMIN et coll 1978 BUCKTON et coll 1967 KWAN & NORMAN 1977 PROSSER 1976) The reduced number of lymphocytes caused by radiation is slow to recover and hardly resumes the pre irradiated value even after one year (HOPPE et coll RUBIN & CASARETT 1968) In a report of patients who were irradiated for seminoma the lymphocyte count only began to approach normal levels at 5 years after irradiation (HEIER et coll 1975) The most remarkable reduction of lymphocyte number is observed when large blood vessels are included within the radiation fields (*CHEE et coll 1974*) indicating that the lymphopenia is primarily due to the

Table 2

Frequencies of chromosome aberrations in cultures of lymphocytes irradiated in vitro

			Chromosome aberrations at indicated incubation time (h)							
Time in culture			44		47		50		55	
Types of culture			Direct	Wash	Direct	Wash	Direct	Wash	Direct	Wa[illegible]
No irradiation		D+R/cell	0/200	0/200	0/200	0/200	0/200	0/200	1/1 000	0/1[illegible]
In vitro irradiation	4 Gy	D+R/cell	74/100	84/100	86/100	99/100	90/100	87/100	9 /100	9[illegible]
			0 90[b]±0 10	0 84±0 09	0 86±0 09	0 99±0 10	0 90±0 09	0 87±0 09	0 9[illegible]±0 10	0 9[illegible]
		X_1Cu-cells	64	76	73	75	65	70	69	69
		Qdr[d]	1 16	1 11	1 18	1 32	1 38	1 24	1 33	1 [illegible]
	6 Gy	D+R/cell	160/100	163/100	171/100	188/100	201/100	196/100	197/100	19[illegible]
			1 60[b]±0 13	1 63±0 13	1 71±0 13	1 88±0 13	2 01±0 14	1 96±0 14	1 97±0 14	1 9[illegible]
		X_1Cu-cells	90	92	95	93	97	95	94	9[illegible]
		Qd[d]	1 78	1 77	1 8	2 02	2 07	2 06	[illegible] 10	[illegible]0

No of dicentrics plus rings/No of analyzed cells

[b] Mean ± standard error

No of cells with unstable chromosomal aberrations which have not attempted a cell division since irradiation

[d] No of dicentrics plus rings/X_1Cu-cells

Table 3

Frequencies of chromosome aberrations in cultures of lymphocytes obtained from patients receiving irradiation

Blood sampling	Interval between end of therapy and examination	Radiation induced aberrations	Chromosome aberrations at indicated incubation time (h)					
			44		47		50	
			Direct	Wash	Direct	Wash	Direct	Wa[illegible]
a	Immediately after the therapy	D+R/cell	71/100	62/100	78/100	94/100	4[illegible]/100	7[illegible]
			0 71[b]±0 08	0 62±0 08	0 78±0 09	0 94±0 10	0 48±0 07	0 7[illegible]
		X_1Cu-cells	36	36	33	49	[illegible]7	40
		Qdr[d]	1 97[d]	1 72	2 36	1 92	1 78	1 8[illegible]
b	24 hours	D+R/cell	15/100	25/100	9/72	30/100	17/67	[illegible]
			0 15±0 04	0 25±0 05	0 13±0 04	0 30±0 06	0 27±0 07	0 [illegible]
		X_1Cu-cells	20	18	18	27	19	[illegible]8
		Qdr	0 75	1 39	0 64	1 11	0 89	1 1[illegible]
c	4 days	D+R/cell	20/100	18/100	25/100	52/100	43/100	[illegible]
			0 20±0 05	0 18±0 04	0 25±0 05	0 40±0 0[illegible]	0 43±0 07	0 [illegible]
		X_1Cu-cells	16	11	20	32	3[illegible]	36
		Qdr	1 25	1 64	1 25	1 63	1 34	1 4[illegible]
d	31 days	D+R/cell	74/100	79/100	75/100	103/100	102/100	[illegible]
			0 74±0 09	0 79±0 09	0 75±0 09	1 03±0 11	1 0 ±0 10	1 [illegible]
		X_1Cu-cells	31	31	30	36	4[illegible]	40
		Qdr	2 39	2 55	2 50	2 86	[illegible] 43	[illegible]
e	6 months	D+R/cell	45/100	54/100	33/100	55/100	[illegible]6/100	[illegible]
			0 45±0 07	0 54±0 07	0 41±0 06	0 55±0 07	0 [illegible]6±0 07	0 [illegible]
		X Cu-cells	28	25	20	[illegible]4	[illegible]5	[illegible]
		Qdr	1 61	2 16	1 65	2 29	[illegible]4	[illegible]

No of dicentrics plus rings/No of analyzed cells

[b] Mean ± standard error

No of cells with unstable chromosomal aberrations which have not attempted a cell division since irradiation

[d] No of dicentrics plus rings/X_1Cu-cells

Table 2 *(cont.)*

		65	
rect	Wash	Direct	Wash
00	0/?00	0/ 00	–
	–	–	–
0/100	190/100	–	–
?80±0 13	1 90±0 14		
	95		
88	2 00		

Table 3 *(cont.)*

		60	
rect	Wash	Direct	Wash
/100	73/100	88/100	67/100
?4±0 08	0 73±0 09	0 88±0 09	0 67±0 08
	40	46	37
	1 83	1 91	1 81
/100	61/100	–	–
45±0 07	0 61±0 08		
	41		
	1 49		
/100	6?/100	60/100	66/100
58±0 08	0 6 ±0 08	0 60±0 08	0 67±0 08
	37	40	4?
41	1 68	1 5	1 57
7/100	186/100	113/100	?03/100
17±0 11	1 86±0 14	1 13±0 11	? 03±0 14
	53	43	58
0	3 51	2 63	3 5
/100	57/100	54/100	5_/100
53±0 07	0 57±0 08	0 54±0 07	0 5?±0 07
	23	4	–
3	? 48	? 25	? 3

killing of mature cells by radiation Further patients with chest irradiation tend to have higher chromosomal aberration frequencies than those with abdominal irradiation (MATSUBARA et coll 1974)

In the present series the peripheral lymphocyte count in irradiated patients did not completely recover to the pre irradiated value even 6 months after the completion of irradiation though slight recovery was evident within 3 weeks

The chromosome aberration yields in lymphocytes of irradiated patients generally increases with increase in accumulated radiation dose (BUCKTON et coll MELLO et coll 1974) The temporary rise in the frequency of radiation induced chromosomal aberrations 2 to 3 weeks after the end of irradiation appears to correspond with the slight recovery of peripheral lymphocyte number This observation may be explained by the release of lymphocytes from the extravascular system which have more chromosomal aberrations due to previous irradiation

In addition there may be other factors to be considered in this temporary rise such as suppressor substance in the serum of patients being irradiated radiation injury of lymphocyte membrane and delay in mitotic progression These factors were analysed by washing the lymphocytes with culture medium and culturing the lymphocytes for various incubation times

No effect of the wash was detected in the culture of non irradiated lymphocytes The lymphocytes irradiated in vitro and in vivo sampled more than 24 hours after the end of irradiation showed an improvement of PHA responsiveness i e mitotic index after washing

However in the lymphocytes sampled immediately after the end of fractionated irradiation no distinct improvement could be identified This observation may be due to either inadequate washing (MANNICK et coll) or fragility of the lymphocytes immediately after high dose irradiation (STEFFEN & MICHALOWSKI 1973)

It has been found that the PHA responsiveness of lymphocytes in patients with advanced malignant tumors is usually defective but is generally improved by washing (MANNICK et coll WHITEHEAD et coll 1976) This improvement of PHA responsiveness in the lymphocytes of irradiated patients after washing with culture medium may be due to the elimination of a suppressor substance in the serum

At least 3 mechanisms have been considered concerning the origin of serum blocking factors (VETTO et coll) (1) classic immunologic enhancement by autologous gamma 2 antibody (2) blocking factor produced by antigen–antibody complexes and (3) effect of a variety of proteins peptides or alpha globulin factors (MCLAUGHLIN et coll)

The possible influence of radiation toxins released into the medium by cell death can be discounted (ILBERY & RICKINSON 1971) since medium collected from leukocyte cultures up to 24 hours after their exposure to 20 Gy could not reduce the subsequent response to PHA of non irradiated lymphocytes from the same donor Further BENJAMIN et coll have reported that no inhibitory factor is present in plasma from irradiated dogs and that the defect appears to reside in the irradiated lymphocytes themselves In the present series peripheral lymphocytes irradiated in vivo recovered their PHA responsiveness following washing with culture medium This restoration of PHA reactivity is associated with an increase in the frequency of radiation induced chromosome aberrations in locally irradiated patients

The concurrent change in Qdr ratio (SASAKI 1971) in the chromosomally aberrant cells indicates that the increase in the aberration frequency and hence the increase in the PHA responsiveness is due to the recovery of PHA reactivity of the more heavily irradiated fraction of cells

In the parallel experiments using in vitro irradiation PHA responsiveness is also improved by washing but not associated with an increase in the chromosomal aberration frequency The reason for this phenomenon perhaps lies with the difference between marked inhomogeneous clinical irradiation and uniform irradiation in experiments in vitro (SHARPE 1969 MATSUBARA et coll)

Mitotic progression in the present experiments was remarkably delayed in the lymphocytes of irradiated patients as evaluated by the chromosomal analysis HEDDLE et coll (1967) were the first to report such radiation induced mitotic delay in lymphocyte culture SHARPE using a mixed culture technique reported that an apparent peak existed in the aberration yields which was delayed by increasing the radiation dose At 400 Gy of in vitro irradiation the duration of delay is reported to be in the order of a few hours

In addition it has been shown that at a higher dose the delay is greater (RICKINSON & ILBERY) and that the cells carrying dicentrics (LLOID et co 1977 STEFFEN & MICHALOWSKI) are more dela in their rate of progress through the first cell cyc than irradiated but aberration free cells In the pr ent series marked delay in mitotic progression w found in the lymphocytes of irradiated patier especially in the lymphocytes with a high Qdr valu

All of these findings indicate that the defect lymphocyte activation in patients receiving po operative radiation therapy and in whom all mal nant tumors are removed is caused by some reve sible change in the lymphocyte membrane direct associated with the radiation exposure rather th by a radiation induced suppressor substance actı on the lymphocyte membrane Glycoproteins in t cell membrane operate as receptors for various le tins and antigens Their sugar portions contrib to the negative charge of the cell membra (MARIKOVSKY et coll 1966 WEISS & SINKS 19 SATO et coll (1977) have shown that roentgen ra can in fact induce a translocation of hyaluronic ac one of the carbohydrates in the membrane from t peripheral zone into the deeper zone The rever shift occurs in the recovery period Disappearan of the effect of washing in lymphocytes obtained months after radiation therapy may be due to t recovery of the injured lymphocyte membrane a reflect a kind of in vivo washing

Previous clinical experience has shown that r gression of malignant tumors occasionally occu following special hemodialysis (PARSONS et co 1974) It would be of great value to determ whether such a phenomenon also occurs by t washing induced restoration of PHA responsiv ness of irradiated lymphocytes

SUMMARY

The peripheral lymphocytes from irradiated pa en generally have a reduced capability to respond phytohemagglutinin (PHA) Whether a relationship ex between PHA responsiveness and chromosome aber tion frequencies was examined by washing the lym cytes with culture medium The results indicate that t defect in lymphocyte activation in patients receiving rad tion therapy was caused by some reversible changes in t lymphocyte membrane directly associated with radia exposure rather than by a radiation induced suppre substance secondarily acting on the lymphocyte me brane

ACKNOWLEDGEMENTS

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Procedures in External Radiation Therapy Dosimetry with Electron and Photon Beams with Maximum Energies Between 1 and 50 MeV

Recommendations by
the Nordic Association of Clinical Physics (NACP)

These recommendations have been produced by the Swedish Association of Radiation Physics and the Nordic Association of Clinical Physics aided by a large number of their members The members of the Topic Group were H Svensson (chairman) L Lindborg (secretary) K A Johansson and N Ulsø Valuable criticism and aid is gratefully acknowledged P Almond A Brahme J Flatby D Harder O Mattsson I Uotila and the Ph D students and teachers from the Radiation Physics Department in Linkoping

The meetings of the working party were supported both by the Swedish and Danish Cancer Society

CONTENTS

Introduction

The International Commission on Radiation Units and Measurements (ICRU) has published general recommendations on dosimetry procedures for photons (ICRU 1969) and for electrons (ICRU 1972) These should preferably be supplemented by national or regional suggestions covering practical details of routine dosimetry procedures and taking into account the particular requirements and provisions of the country and region Local recommendations have been prepared for the United Kingdom (HPA 1969 1971 1975) the USA (SCRAD 1966 1971 AAPM 1975) West Germany (DIN 6809 1976 DIN 6800 1975a b) and the Nordic countries (NACP 1972)

The present report contains a revised Nordic protocol Several reasons have motivated this revision After publication of the first protocol several reports have been published giving new data on various effects which can change the factors used with ionization chamber dosimetry The SI units for the radiologic quantities should be applied Another important reason is that the former Nordic recommendations were mainly based on investigations with betatrons while within the Nordic countries now several other kinds of accelerators are used (standing wave and travelling wave linear accelerators and microtrons) with usually different properties which have to be considered Improved concepts for stating beam quality and beam uniformity etc are therefore introduced in the present report Similar revisions are being carried out by the ICRU and the AAPM

Differences in the absorbed dose determination using the methods given in NACP (1972) compared to methods given in the present protocol can be as large as 5 per cent in extreme cases It is recommended that the new protocol will be adapted at various centres as soon as new calibrations of ionization chambers have been achieved

In the present report *shall* means compulsory for compliance with this report and *should* means strongly recommended

These recommendations do not include the complete procedures for dosimetry of electron beams in the range of 1 to 10 MeV mean electron energy at the phantom surface where plane paralle bers shall be used Such procedures will plained in a supplement to this protocol to lished in the near future

The aim of the recommendations is to gi pital physicists a code of practice to be f at radiation therapy centres in Denmark I Iceland Norway and Sweden so as to sec formity in dosimetry procedures They co tests and measurements both when dosim first performed with a new therapy apparatu the continuous supervision necessary to ens the dosimetry is properly employed in r therapy In weighting high accuracy and t strictness against practical usefulness the la been given more emphasis so that the pro may be performed easily at all therapy centr

Principal features The dosimetric metho ing air ionization chambers is still recommen linking the national standard to the local ref mainly in view of their general availability s ity and precision For the radiation quant concern here the ionization chambers shall brated at national radiation standards labo in ^{60}Co-γ ray beams

Since the formerly used C_E and C_λ value only valid for air equivalent and water equ chamber walls respectively a new proced the determination of absorbed dose is mended The procedure is based on a deriv sorbed dose ionization chamber factor (N_D) the ratio between the mean absorbed dose t the ionization chamber cavity and the scale r N_D is derived from a calibration in air in a ray beam The absorbed dose to water at radiation qualities is then obtained as the pro meter readings N_D stopping power ratios a turbation factors Stopping power ratios an turbation factors are both listed The latter an for chambers of either air or water equivale terials the equivalence of concern being the erty of generating secondary electrons Wa air equivalent materials are recommended ionization chambers at least for reference ments

Table 1
Energy quantities for specifying radiation beams

Quantity	Use	Determination		Energy range
E	On accelerator console	$E \approx E_{0}+\Sigma S_{\text{ni}}\,\Delta Y_i$	eq (1)	$1\ \text{MeV}\leq E \leq 50\ \text{MeV}$
E_{0}	Specification of absorbed dose distribution	$E_{0}=C+C_2R+C_3R$ $C=0.72$ MeV $C=1.98$ MeV cm^{-1} $C=0.0025$ MeV cm^{-2}	eq (2)	$1\ \text{MeV}\leq E \leq 50\ \text{MeV}$
E	As reference to dosimetric constants	$E_0=C\,R_{50}$ $C=2.33$ MeV cm	eq (3)	$5\ \text{MeV}\leq E_0\leq 30\ \text{MeV}$

The increasing number of accelerators of different types and often quite different beam qualities necessitates a simple unified comparison of the quality of one beam with another The main therapeutic and physical properties of the depth dose distribution of the beams are therefore characterized by new parameters not used in NACP (1972)

Energy determination at accelerators

A knowledge of the radiation quality is necessary because stopping power values and perturbation factors recommended here for ionization chamber dosimetry are energy dependent and because standardized depth dose tables may be used for accelerators similar in construction provided the energy is determined in a uniform manner (SVENSSON & HETTINGER 1971 SVENSSON 1971) Furthermore quality parameters may be desired for the comparison of one beam with another Depending on the parameter of interest different energy *quantities are recommended* For electron beams it is recommended to use a therapeutic range to describe the radiation quality in irradiation procedures

Electron beams

Energy quantities The intrinsic accelerator beam i e the electron beam just before the exit window of the accelerator but after beam handling magnets and energy defining slits has a certain *energy distribution* This can be characterized by its maximum energy ($E_{m\,a}$) its most probable energy $E_{p\,a}$ its mean energy E and its energy spread Γ_a the index a stands for accelerator As the beam passes through the exit window and different materials from the exit window to the phantom surface the energy will decrease and the energy spread increase

Therefore analogous energy quantities can be defined for the phantom surface index o and for any depth z in the phantom index z These different electron energy quantities are recommended and are summarized in Table 1 and Fig 1 Their principal uses are

$E_{p\,a}$ (the most probable energy in front of the accelerator window) For a given accelerator beam setting different beam scattering foils or decelerators are often used However the energy instruments are usually constructed to give a measure of the electron energy of the intrinsic beam Therefore it is recommended that the energy indication meter and the energy selection setting on the console desk be calibrated in an energy quantity which is independent of the materials in the beam $E_{p\,a}$ should be the energy quantity to use for this purpose

$E_{p\,0}$ (the most probable energy at the phantom surface) To indicate the energy of an absorbed dose distribution $E_{p\,0}$ shall be used The reason is that *this energy quantity is well related to the practical* range R_p which generally is used for energy determination $E_{p\,0}$ is in most accelerator facilities 1 to 2 MeV lower than $E_{p\,a}$ for energies below some 20 MeV This difference may increase with energy

$\bar{E}_0$ (the mean energy at the phantom surface) In absorbed dose measurements with an ionization chamber the relevant stopping power ratios and perturbation factors must be known These are in this *protocol given as a function of* $\bar{E}_0$ and the depth of the chamber in the phantom (Tables 5 7) [In NACP (1972) the absorbed dose conversion factors (C_E) were correlated to the mean energy at a phantom depth a quantity estimated from the relation

In this equation E_0 was approximated by E_{p0}. In the present protocol it is considered that E_0 may be a few MeV lower than E_{p0} and therefore that a determination of E_0 should be made.]

Energy determinations. E_{p0} should be determined by analysing the central axis depth dose curve making use of the empirical relation between the practical range in water, R_p, and E_{p0} (Table 1). In the energy range 7 to 20 MeV eq. (2) gives within ±1 per cent the same E_{p0} as the linear equation recommended in NACP (1972), i.e. $R_p = E_{p0} \cdot 0.52 - 0.3$. Outside this range the difference increases. For the energy range 1 to 50 MeV the relation recommended here (eq. 2) fits experimental (MARKUS 1964, HARDER & SCHULZ 1971) and calculated data (SELTZER et coll. 1977) to within 2 per cent.

The practical range, R_p, in eq. (2) (Table 1) is defined as the intersection depth of the tangent through the steepest point (inflection point) of either a depth absorbed dose curve or a depth ionization curve and the photon background (Fig. 2). The curves should be measured as described on page 17 making use of the concept of the effective point of measurement.

Depth absorbed dose and depth ionization curves in a water phantom give within about 1 to 2 mm the same value of R_p (SVENSSON & HETTINGER 1971). For the determination of E_{p0} the measurement of depth ionization curves is recommended. Above 10 MeV both cylindric and plane parallel chambers could be used but below about 10 MeV only plane parallel chambers should be used as they have the best defined effective point of measurement. For energies above 10 MeV a water phantom shall be used, while below 10 MeV either a water or a plastic phantom can be used. Large field sizes must be used, i.e. ≥120 mm × 120 mm for energies up to 20 MeV and ≥200 mm × 200 mm above that energy. The source-surface distance (SSD) should be ≥1 m. With some plastic phantoms the relation between R_p in water and in plastic may be calculated from the formula (MARKUS 1961, DIN 1976):

$$\frac{R_{p,H_2O}}{R_{p,pl}} = \frac{\varrho_{pl}}{\varrho_{H_2O}} \times \frac{\left(\frac{Z}{A}\right)_{eff,pl}}{\left(\frac{Z}{A}\right)_{eff,H_2O}} = k \qquad (4)$$

In this equation $(Z/A)_{eff} = \Sigma f_i (Z_i/A_i)$, where f_i is the fraction by weight of the constituent element of atomic number Z_i and the relative atomic mass A_i. ϱ is the density. Index pl stands for plastic. Eq. (4) may be used for materials with $(Z^2/A)_{eff} < 4$ (DIN 1976); values of some materials are given in Table ...

E_0 should be determined from the empirical eq. ... (Table 1) relating it to the half value depth, R_{50}, defined as the depth of the 50 per cent depth absorbed dose in a water phantom (Fig. 2). The ... eq. (3) should be used in the energy range ... MeV. The relation between R_{50} and E_0 outside ... range should be taken from Table 5 and Fig. ... Eq. (3) is strictly valid only for an infinite SSD but may also be used for SSD down to 1 m ... energies up to approximately 20 MeV (Fig. ...

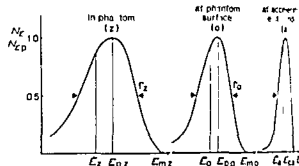


Fig. 1. The distribution of electrons in energy in front of the accelerator window (a), at the phantom surface (o) and at the phantom depth (z). The ordinate shows the differential distribution in energy of the one directional plane fluence N_E normalized to its value at the most probable energy, N_{Ep}.

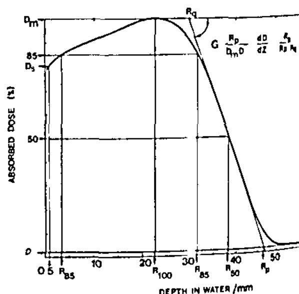


Fig. 2. The depth absorbed dose distribution with definitions of the parameters used in the text. D_m is the level of maximum absorbed dose, D_s is the surface dose measured at 0.5 mm depth, D_x is the photon background, G is the dose gradient, R_{100} the depth of dose maximum, R_{85} is the therapeutic range, R_{50} the half value depth and R_p the practical range. (From BRAHME & SVENSSON 1979.)

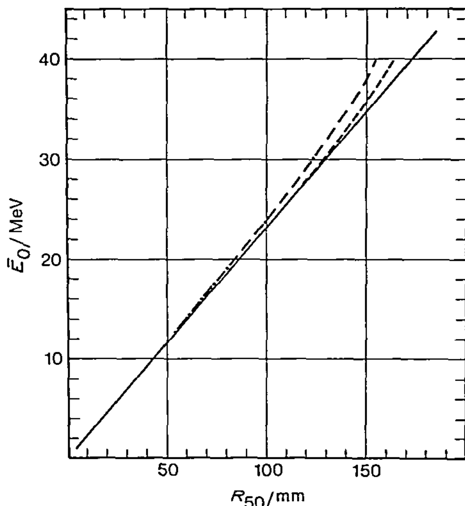


Fig 3 The relation between R_{50} and $\bar{E}_0$ for large field sizes The solid line is valid for R_{50} determined from beam axis depth absorbed dose curves with SSD=∞ the broken line for beam axis depth absorbed dose curves with SSD=1 m and the dash-dotted line for beam axis depth ionization curves with SSD=1 m

Above that energy inverse square law corrections should be performed R_{50} should be measured with large field sizes and should be determined from a depth absorbed dose curve but may be evaluated from a depth ionization curve and the graph in Fig 3

$\bar{E}_{p,a}$ should be calculated according to eq (1) in Table 1 The calculation means that the energy losses of the electrons in all scattering materials in the radiation beam e g window scattering foils transmission chambers and air must be added to the electron energy $E_{p,0}$ The thickness of the various materials $\Delta\lambda_i$ which the beam passes from the inner side of the tube window to the phantom surface must be known as well as the collision stopping power of each material $s_{coll,i}$ Materials in the beam very near the phantom surface should if possible be removed in the measurements of $E_{p,0}$ as eq (1) gives an incorrect estimate of $E_{p,a}$ with materials near the phantom surface

Table 2
Some characteristics of phantom materials

Phantom material	Composition	ϱ g cm	$\left(\frac{Z}{A}\right)_{eff}$	k
Water	H O	1	0 555	1 000
Polystyrene	C H	1 05	0 538	1 018
Perspex	C H O_2	1 18	0 540	1 148
A 150	see ICRU (1977)	1 1[illegible]	0 548	1 106

Photon beams

Energy determinations For a proper choice of stopping power ratios and perturbation factors in photon beams a measure of the photon beam quality can be estimated from depth ionization measure

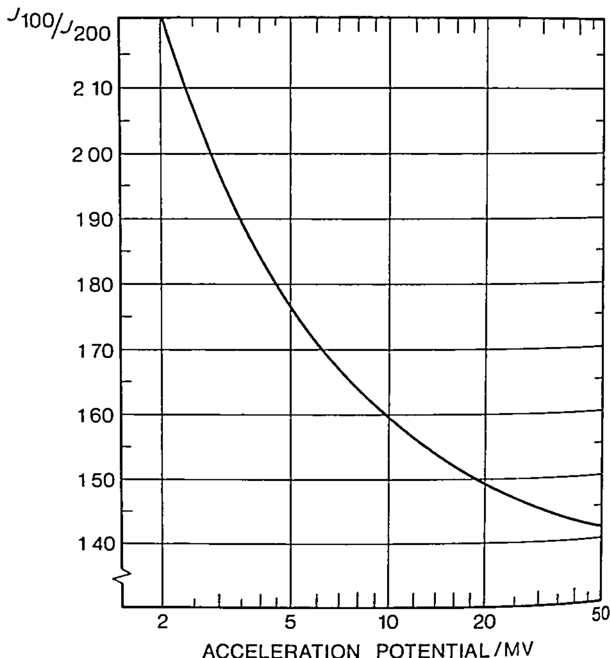


Fig 4 The ratio J_{100}/J_{200} ($=D_{100}/D_{200}$) as a function of the accelerating potential (or $h\nu_m$) is shown for a SSD = 1 m and a field size of 100 mm × 100 mm For accelerators with the same accelerating potential the ratios differ from beams with different targets and flattening filters and are as a rule smaller for betatrons than for linear accelerators and microtrons All maxim… 20 MV are from betatrons while those below 20 MV are ta… from linear accelerators J_{100}/J_{200} is recommended as mean d… for (s … n)

ments (BRAHME & SVENSSON 1979) The ionization at the depth of 100 mm and 200 mm in a water phantom is measured for a field size of 100 mm × 100 mm and an SSD of 1 m and the ratio J_{100}/J_{200} determined The ratio is stronger related to the mean photon energy than the maximum photon energy Therefore if the ratio is calculated from published data and plotted against the maximum photon energy a rather large spread is found (Fig 4) The J_{100}/J_{200} method is recommended for estimation of the photon beam quality rather than measurem… of the half value depth R_{50} as the values of … depend upon the contamination of electrons in th… peak absorbed dose of the photon depth dose cur…

The maximum photon energy $h\nu_m$ in the ph… beam can be estimated with two different meth… When both photon and electron beams are avail… from the same accelerator the maximum photon en… ergy may within 1 or 2 MeV be approxima… of th… $E_{p,0}$ The energy meter on the accelerator is u…

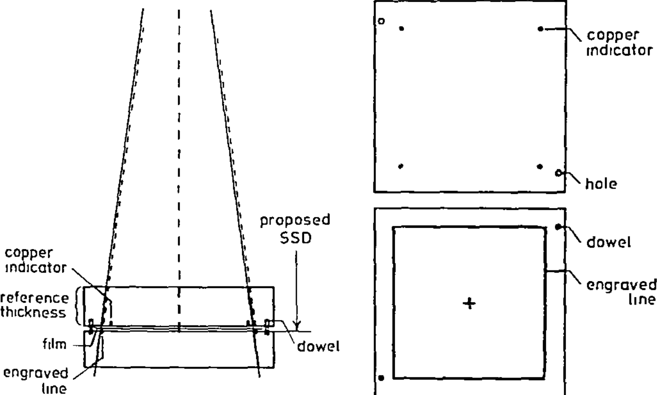


Fig 5 Polystyrene phantom to be used for beam alignment check A small misalignement is indicated in the figure as the solid and broken lines do not coincide Light beam — Radiation beam - Beam axis

designed to give a measure of the electron energy before the exit window and photon target The meter can therefore be calibrated against $E_{p,a}$ for the electron beams (see page 5) Alternatively a method based on (γ n) threshold measurements may be used (NACP 1972) This method makes a direct calibration of the energy instrument in the maximum photon energy $h\nu_m$ possible The maximum photon energy has conventionally been used to specify depth dose distributions

Recent investigations have shown that the shape of the depth absorbed dose curves and the mean energy for photon beams may be more dependent on the construction of target and flattening filter than on a change of a few megavolts in accelerating potential i e in change of E_m (PODGORSAK et coll 1975 NAHUM 1978) Therefore it is not considered necessary to carry out determinations of $h\nu_m$ using the methods mentioned neither for the purpose of dosimetry nor for the specification of depth absorbed dose curves The J_{100}/J_{200} method is uncertain for the estimate of the maximum photon beam energy but is well related to the mean photon energy and should therefore be used for dosimetric procedures The ionization ratio is recommended as a beam quality parameter for use in choosing the stopping power ratios necessary for ionization chamber dosimetry (Table 6)

Geometric considerations

The position of the radiation beam must be defined and indicated to be able to perform accurate radiation therapy In this section some simple procedures are given The discussions are limited to rectangular unmodified beams only e g without wedges or blocks

Beam alignment

The beam alignment shall be checked on the installation of a new therapy unit The manufacturer should be held responsible for any adjustment required before the accelerator is handed over for routine treatments

The proper alignment of the different types of beam axes (collimator rotation axis geometric beam axis radiation beam axis and light beam axis as defined in the Appendix) shall be checked before the absorbed dose distributions are determined Practical beam alignment procedures have been detailed in

Table 3
Depth of reference plane

Type of radiation	$h\nu_m$ or E	Depth of reference plane
Photons	1–<10 MeV	50 mm
	10– 50 MeV	100 mm
Electrons	1– <5 MeV	Peak absorbed dose
	5–<10 MeV	Peak absorbed dose or min 10 mm
	10–<20 MeV	Peak absorbed dose or min 20 mm
	20– 50 MeV	Peak absorbed dose or min 30 mm

the case of electron and photon beams by HPA (1970) AAPM (1975) and may be consulted when the checks are carried out Also the International Electrotechnical Commission (IEC) has a working group dealing with such procedures

An alternative method to test the position of the light beam in comparison with the radiation beam appears in Fig 5 The test should be carried out in the following order The bottom sheet of the phantom is placed perpendicular to the collimator axis with the front surface at the SSD in use The light beam size and position shall coincide with the engraved line on the bottom sheet The numerical field size shall be noted A film in a light tight cover is placed on the bottom block and a top block with 4 metal indicators is placed with care on the film without moving the bottom block The thickness of the top block should be approximately equal to the reference depth (Table 3) After irradiation 4 dots on the film are observed from the indicators These dots should be used for comparison of the position of light and radiation beam The uniformity index the radiation field size and the physical penumbra may also be determined from the same film The beam and the collimator axes shall agree within 2 mm and the position and size of the radiation beam and light beam shall agree within 2 mm at an SSD of about 1 m

Depth of the reference plane

Recommended depths for the reference plane for various radiation qualities are given in Table 3 For electron beams the depth of the absorbed dose maximum is recommended as the reference plane instead of a fixed depth due to the peaked depth absorbed dose curves for some low energy electron beams However for some accelerators and beam sizes the maximum absorbed dose can occur at very small depths due to electrons scattered from for instance collimators In those cases the depth of the reference plane should be taken as the minimum values given in Table 3

Uniformity of the beam

A useful measure of the beam uniformity is the uniformity index (see Appendix) This index should exceed 0 80 in the reference plane for the absorbed dose at field sizes larger than 100 mm × 100 mm for both photon and electron beams In addition the beam uniformity should be such that the absorbed dose at any point in the reference plane should not exceed 103 per cent of that at the reference point For accelerator beams the physical penumbra (see Appendix) shall not exceed 8 mm at an SSD of about 1 m Some accelerators are overflattened at small phantom depths in order to achieve a good uniformity index at large depths Then for any plane parallel to the reference plane the absorbed dose of an arbitrary point in that plane should not exceed [illegible] per cent of its value on the beam axis

The radiation beams should as minimum requirements fulfil the values given but each hospital physicist should work for achieving as good beam uniformity as possible generally aiming at an absorbed dose variation within the target volume of less than ±5 per cent (ICRU 1976)

The uniformity the radiation field size and the physical penumbra may be measured in various ways Photographic film in a polystyrene phantom offers the advantages of high spatial resolution simplicity of handling short irradiation times and above all the fact that it will lead to simultaneous recording of the entire radiation field With some care the figures given for absorbed dose uniformity may be equated to the same figures obtained for net film blackening A disadvantage of the film method is however that it is best used with an automatic or semi automatic density plotter With the film method the beam uniformity should be investigated by means of photographic film in a light tight cover of regular thickness but not of a radiation fluorescent material industrially pre wrapped film may be used The film should be exposed in a polystyrene phantom (Fig 5) and the absorbed dose should be about 1 to 2 Gy (a typical treatment absorbed dose) so as to minimize the influence of initial perturbations in accelerator beams and of shutter movement in ^{60}Co-γ beams Before irradiation accelerators should have been run under operating conditions for

a sufficient period of time to effect a proper warm up The relation between film blackening and the absorbed dose depends critically on the develop ment procedure used parameters of importance are type of film and developer development time and temperature All these should be combined to pro duce a linear relationship between blackening and absorbed dose over a large range The aperture dia meter of the density reader should be selected to exert negligible influence on the spatial pattern re corded The variation in film density on a homo geneously exposed film should be less than ±2 per cent only a few types of film seem to meet these requirements (RASSOW et coll 1969 DUTREIX 1976) The film density versus absorbed dose should be checked regularly and at least every time a film batch or developer is changed

Alternatively a semi conductor detector or a small ionization chamber (i e diameter of not more than 5 mm) could be used for traversing the beam in the reference plane in a water tank for gantry angles at 90° 270 and possibly 0° from the vertical position This method will produce higher precision than the film method provided that in connection with accelerators a monitor probe is used to correct for output fluctuations The monitor probe should be placed either outside the region of interest (the 50 per cent isodose curve) or in the centre of the beam in which case the shadowing effect must be taken into account when evaluating the results For other gantry angles the beam could be investigated by means of a polystyrene block with holes for the de tector (NAYLOR & CHIVERALLS 1970) The ab sorbed dose distribution in the reference plane may then be equated to the ionization current distribu tion

Determination of absorbed dose at reference points

The absorbed dose determination shall be made by air ionization chamber measurements performed by a qualified person A new determination shall be made when new conditions of irradiation are em ployed The determination shall be confirmed with an independent dosimetric method for instance calo rimetry or ferrous sulphate dosimetry and even less accurate methods such as those based on solid state dosimetry may be used Both the absorbed dose determination and confirmation shall be performed before irradiation of patients In connection with these absorbed dose determinations a constancy check procedure shall be incorporated

Ionization chambers and electrometers

Reference instrument Each radiation therapy centre shall have at least one local reference (stand ard) ionization chamber together with an electro meter selected as a reference instrument for calibra tion of field ionization chambers and other dose meters A reference chamber may also be used for the first calibration of a new therapy machine How ever due to good stability of modern electrometers a reference electrometer may be used also for other purposes The reference chambers shall be cali brated at a standardizing laboratory particularly for ^{60}Co γ rays in air The reference electrometer shall also be calibrated at a standardizing laboratory The calibration is preferably carried out separately for the ionization chamber and the measuring assembly The chamber should be re calibrated at a standardiz ing laboratory at least once every 2 years A longer interval can be acceptable for the electrometer if its stability can be checked in an independent way

The response of the reference instrument (ioniza tion chamber and electrometer) should be checked at least quarterly against a suitable radioactive source at the radiation therapy centre e g ^{60}Co γ source (half life 5 27±0 01 year) Any change in re sponse of the reference instrument of more than 1 per cent revealed by the constancy checks should lead to a thorough investigation of instrument and subsequent re calibration at the standardizing lab oratory

A cylindric chamber with an air volume of 100 to 1 000 mm³ should be used as reference ionization chamber If the chamber is to be used for calibra tions of field instruments under conditions other than those at the standardizing laboratory (for in stance in a phantom) it is essential that the air vol ume diameter in the chamber is between 4 and 6 mm and has a length of less than 25 mm The wall of the chamber should be homogeneous graphite or tissue/ water or air equivalent material The thickness of the graphite chamber wall should preferably be about 0 5 mm as the perturbation factors reported here have been determined with this wall thickness (ALMOND & SVENSSON 1977 JOHANSSON et coll 1977) The wall thickness is of less importance for water equivalent chambers It is important that the material of the build up cap is the same as that of the wall In order to avoid mistakes the build

should be clearly marked and shall belong to a certain ionization chamber The central electrode should be made of the same material as the wall and should preferably not be too massive Leakage current radiation induced current and current generated in the stem must be negligible The ratio between the ionization currents measured at positive and negative polarizing potential shall be checked and should be less than 1 005 for any radiation beam quality This polarity effect increases with decreasing electron energy and should therefore for a cylindric ionization chamber be determined at various depths in a phantom for a beam of about 10 MeV mean electron energy at the surface

Field instruments The ionization chamber used for the assessment of absorbed dose must fulfil certain requirements A cylindric ionization chamber should be used at all photon radiation qualities and at electron beams with a mean energy at the phantom surface E_0 above 10 MeV A plane parallel chamber should be used at electron energies E_0 equal to or less than 10 MeV A supplement of this protocol will treat the measurement procedure used for the plane parallel chamber

The cylindric field chamber should have dimensions like the reference chamber Chambers with unknown wall central electrode material and thickness or with a thin layer of inner conducting material should be subjected to a special response test i e be calibrated against the reference chamber for all radiation qualities used Leakage current radiation induced current and current generated in the stem must be negligible

The response of the field instrument should be checked at least quarterly against a suitable radioactive source or against the reference instrument Any change in sensitivity of the field instrument of more than 1 per cent revealed by the constancy check should lead to a thorough investigation of the instrument and subsequent re-calibration against the reference instrument

Ion recombination The losses due to ion recombination are generally less than 1 per cent for continuous radiation or pulsed radiation of an absorbed dose to air in the chamber cavity per pulse of 1 mGy or less if the collection voltage is higher than 300 V for the type of chamber mentioned above If the losses are less than 1 per cent re-combination correction will not always be necessary Correction for recombination losses can be made either by calculation (BOAG 1966 ICRU 1964) or by measurements (ICRU 1972) For pulsed radiation beams the measured charge is plotted against the inverse of the polarizing voltage in the region of losses below 5 per cent The released charge is determined by l extrapolation in this plot to infinite polarizing vol

Electrometer A precision low current meas electrometer shall be used The electrometer is ally based on a high gain amplifier with a low l age current working either in a charge/voltage m or in an integrating Townsend balance mod digital display is usually preferable A solid s electrometer may be built with either field e transistors (MAUDERLY & BRUNO 1966) or l varactor bridge input amplifier (JOHANSSON et 1972) The scale on the electrometer should pr ably be marked in coulomb and ampere The trometer should have a good long term stability a number of years

The instruments shall be constructed so a minimize the effect of external electrostatic netic and electromagnetic fields However strong electromagnetic field that exists in around some accelerators may affect the readir the instrument Special attention is needed to er that this does not occur However large mis in the dosimetry due to these effects will be covered as 2 independent methods shall be use absorbed dose determination at an accelerator

Calibration at national standards laboratori

The local reference chamber and measuri sembly shall be calibrated at national standards oratories in a beam of ^{60}Co-γ rays The calibra of the ionization chamber shall be made in air distance of about 1 m from the source to the ch ber centre and at a field size of about 100 m 100 mm The cylindric chamber should have an ditional cap of water or air equivalent material chambers of water and air equivalent walls res tively to assure electron equilibrium The t thickness of wall and additional material shoul 0 45±0 05 g cm^{-2} If a perspex cap is used i of the recommended materials and if this pro is followed the systematic error introduced wo less than 1 per cent (ALMOND & SVENSSON 1 JOHANSSON et coll 1977)

International recommendations now exist f mary standards laboratories to derive air k from exposure measurements using agreed conversion factors The relation between air k (K_{ai}) and exposure (X) is (ICRU 1971)

$$K_{air}(1-g)=X\,W/e \qquad (5)$$

where

g =the fraction of the energy of the secondary charged particles lost to bremsstrahlung in air (g is at ^{60}Co γ rays close to 0 4 per cent BOUTILLON 1977)

W/e=mean energy expended in air per ion pair formed and per electron charge (W/e is equal to 33 85 J C^{-1} ICRU (1979))

The laborious use of the SI units for exposure (C kg^{-1}) in some applications can then be overcome from the use of air kerma (unit Gy) The Nordic standards laboratories will be able to provide air kerma calibration factors (N_K) at ^{60}Co γ beams defined by

$$N_K=\frac{K_{air,c}}{M_c} \qquad (6)$$

where

$K_{air,c}$=kerma in air at the centre of an ionization chamber in the absence of the chamber at the calibration radiation quality in Gy

M_c =meter reading at calibration corrected for temperature pressure humdity etc in C or div

(General re combination corrections should not be necessary to perform at the calibration in ^{60}Co γ beams with the types of chambers recommended in this protocol)

Derivation of absorbed dose ionization chamber factor

The mean absorbed dose to air D_{air} inside the air cavity of the ionization chamber has to be evaluated from the observed air kerma K_{air} if as suggested in this report the Bragg Gray relation is to be used for determination of absorbed dose to water The following relation between $D_{air,c}$ and K_{air} is used

$$D_{air,c}=K_{air,c}(1-g)\ k_{att}\ k_m \qquad (7)$$

where

k_{att}=attenuation and scattering factor correcting for attenuation and scattering in the ionization chamber material at the calibration in the ^{60}Co-γ beam

Table 4

k_{att} and k_m values for cylindric ionization chambers of sizes recommended in this protocol for different materials of the wall and cap combination The values are expected to be dependent on the shape size and electrode design

Chamber wall and cap material	k_{att}	k_m
Air equivalent	0 990	1 000
Graphite	0 990	0 991
Tissue equivalent (A 150)	0 990	0 963

k_m =chamber material dependent factor correcting for the lack of air equivalence of the ionization chamber material

The correction factors k_{att} and k_m have been discussed extensively in the literature recently e g by ALMOND & SVENSSON (1977) and JOHANSSON et coll (1977)

The symbol A with various indices is often used instead of k_{att} and k_m different authors giving different meaning to the symbol The factors recommended (Table 4) are those given by JOHANSSON et coll (1977)

The absorbed dose to air ionization chamber factor N_D is derived from eqs (6) and (7) as

$$N_D=\frac{D_{air}}{M_c}=N_K(1-g)\,k_{att}\,k_m \qquad (8)$$

N_D could be derived for any ionization chamber but the values in Table 4 are only valid for chambers described on p 11 N_D could be stated by national standards laboratories or be calculated at the hospitals If given by standards laboratories the factors k_{att} and k_m must be clearly stated in the protocol

(N_D is related to an exposure calibration factor (N_X) through the formula

$$N_D=N_X\,k_{att}\,k_m\frac{W}{e}\,k_1 \qquad (9)$$

where

k_1=1 00 with N_X in C kg^{-1} C^{-1}
k_1=2 58 10^{-4} with N_X in R C^{-1})

Absorbed dose determination at the reference point

For all photon radiation beams with maximum energies above 1 MeV and all electron radiation

Table 5

Recommended values of $(s_{w,air})$ and p for electron radiation at the reference point in a water phantom The absorbed dose maximum is assumed to be at the minimum reference depths given in Table 3 The $(s_{w,air})$ shall be taken from Table 7 if dose maximum and therefore the reference point is situated at larger depths than given in Table 5 The p may be taken from Table 5 as it is not critically dependent on the depth

E (MeV)	R_{50} (absorbed dose measurement) SSD=1 m (mm)	R_{50} (ionization measurement) SSD=1 m (mm)	Minimum reference depth (mm)	$(s_{w,air})$	p *
1	3	3	2	1 144	–
2	7	7	4	1 137	–
3	12	12	6	1 122	–
4	16	16	8	1 108	–
5	21	21	10	1 097	–
6	25	25	10	1 078	–
7	30	30	10	1 061	–
8	34	34	10	1 048	–
9	38	38	10	1 036	–
10	43	43	20	1 053	(0 975)
12	51	51	20	1 033	0 980
14	60	59	20	1 018	0 985
16	68	67	20	1 006	0 985
18	78	76	20	0 997	0 990
20	86	84	30	1 001	0 990
22	94	92	30	0 993	0 995
25	107	104	30	0 981	0 995
30	128	123	30	0 965	0 995
35	146	140	30	0 952	0 995
40	163	154	30	0 942	1 000
45			30	0 934	1 000
50			30	0 930	1 000

Values from BERGER et coll (1975) with cut-off energy Δ= 15 keV

Values from JOHANSSON et coll (1977) for a cylindric ionization chamber with diameter 5 mm of tissue/water equivalent or air (graphite) equivalent material

A plane parallel ionization chamber is recommended for E_0<10 MeV

beams with E_0 above 10 MeV the absorbed dose at the reference point should be determined in a water filled polymethylmethacrylate or polystyrene phantom with outer dimensions at least 0 3 m × 0 3 m × 0 3 m The thickness of the phantom walls oriented towards the radiation source should be 5 mm or less The water filling should be at least 0 25 m If the distance between the edge of the beam and the edge of the phantom becomes *less than 50 mm at the en*trance surface a larger water phantom should be used so that the distance will never be less than 50 mm The ionization chamber should be protected during the water measurement by a tube with a wall thickness of 1 mm manufactured from polymethylmethacrylate This tube should be attached to a holder that can be adjusted for measurements at various depths The symmetry axis of the chamber must be positioned in the reference plane

For electron energies in the range $1 \le E_0 \le 10$ MeV the absorbed dose in the reference point should be measured in a solid phantom A plane parallel ionization chamber should be used (see supplement to be published)

The effective point of measurement for a cylindric ionization chamber is *displaced from the centre of* the chamber towards the radiation source (DUTREIX & DUTREIX 1966 HETTINGER et coll 1967) However for electron radiation the centre of the cylindric chamber may be placed at the reference depth as this is situated at a dose plateau or at least on a slow varying part of the depth ionization curve For photon radiation the chamber centre shall be placed at the reference depth due to convenience In this case correction factors are included in the total perturbation factors below in order to correct for displacement

Measurements should be made for all combinations or irradiation conditions A horizontal or vertical beam direction may be employed With wedge fields the edge of the wedge should be placed parallel to the symmetry axis of the ionization chamber *The measurements* should be made at the two possible 180° different orientations of the wedge and the average result should represent the absorbed dose

The Bragg Gray equation is recommended for the determination of the absorbed dose at the reference point in the water in the absence of the chamber at the user s radiation quality $D_{w,u}$ Thus

$$D_{w,u} = \bar{D}_{air,u}\, p_u\, (s_{w,air})_u \qquad (1$$

where

$\bar{D}_{air,u}$ = mean absorbed dose to air in the cavity of the ionization chamber measured in water at the user s radiation quality in Gy

$(s_{w,air})$ = mass stopping power ratio water to air at the reference point at the user s radiation quality

p_u = total perturbation factor including corrections for lack of water equivalence in the

Table 6

Recommended values of $(s_{w,air})_u$ and p_u for photon radiation at the reference point in a water phantom. The variations of the stopping power ratios with depth for depths beyond the absorbed dose maximum are considered to be negligible (NACP 1975)

Radiation beam quality	J_{10}/J_{20}	$(s_{w,air})_u$ *	$p_{u,graphite}$ **	$p_{u,water}$
Co-γ	1.97	1.150	0.970	0.990
4 MV	1.84	1.145	0.970	0.990
6	1.71	1.140	0.980	0.990
8	1.63	1.135	0.980	0.995
10	1.59	1.125	0.985	0.995
12	1.56	1.120	0.985	0.995
14	1.54	1.115	0.985	0.995
16	1.52	1.110	0.985	0.995
18	1.50	1.105	0.985	0.995
20	1.49	1.105	0.985	0.995
22	1.47	1.100	0.985	0.995
25	1.46	1.095	0.990	0.995
30	1.45	1.090	0.990	0.995
35	1.44	1.080	0.990	0.995
40	1.43	1.075	0.990	0.995
45	1.43	1.070	0.990	0.995
50	1.42	1.065	0.990	0.995

* Values from ICRU (1969)

** p_u values from JOHANSSON et coll. (1977) for a cylindric ionization chamber with diameter 5 mm

ionization chamber material at the user's radiation quality, perturbation of the fluence due to the insertion of the air cavity and location of the effective point of measurement of the cylindric chamber due to the curved ionization chamber wall, only for photon beams.

With the assumption that

$$N_D = \frac{D_{air,c}}{M} \approx \frac{D_{air}}{M}$$

the absorbed dose at the reference point in water is obtained from

$$D_w = N_D M_u p_u (s_{w,air})_u \qquad (11)$$

where

M_u = meter reading at user's quality, corrected for temperature, pressure, recombination, humidity etc. in C or div.

This is the essential equation for practical dosimetry work. p_u factors for different radiation beam qualities and ionization chamber materials are given in Tables 5 and 6. $(s_{w,air})_u$ values are found in Table 7.

In the symbol $(s_{w,air})$ is not specified the type of stopping power ratio. Two different sets of stopping power ratios should strictly have been used, one for a chamber of air equivalent walls and another for a chamber of water equivalent walls. In the first case Harder's extended Bragg-Gray cavity theory (HARDER 1965) is to be used, which means that it is assumed that a delta ray equilibrium exists in the air cavity due to the air equivalent wall (i.e. the energy carried into the cavity by delta rays is balanced by energy carried out by delta rays). $(s_{w,air})_u$ should then be the collisional mass stopping power ratio water to air. In the second case Spencer-Attix theory is to be used. The distribution of the electron fluence down to a certain cut-off energy must then be known at the point of measurement in the water phantom and the restricted stopping powers should be used for this distribution. The cut-off energy is dependent on the size of the chamber. However, in electron beams the two sets of data differ with less than one per cent when using the mean electron energy calculated according to BRAHME (1975) for determination of the collision stopping power ratios in the Bragg-Gray Harder theory and when using BERGER et coll. (1975) electron fluence distribution to determine the mass stopping power ratio in Spencer-Attix theory. Furthermore, experiments in both electron and photon beams with air equivalent and water equivalent chambers show that the same stopping power data within about one per cent could be used for the two types of walls (JOHANSSON et coll. 1977). Therefore, for simplicity, only one set of $(s_{w,air})_u$ is recommended. The $(s_{w,air})_u$ from BERGER et coll. (1975) are recommended for electron radiation and those from the ICRU (1969, Table A 3) for photon radiation.

Illustrative example

Calibration at standards laboratory. The national standards laboratory has performed a calibration of the ionization chamber, which has a diameter of 5 mm and a graphite wall of about 0.5 mm. A build-up cap of graphite was used at the calibration and the thickness of wall and cap together was 0.45 g cm⁻². The following information on the ionization chamber calibration factors are given in the calibration certificate.

The air kerma calibration factor N_K obtained for the ionization chamber in the ^{60}Co-γ ray beam at the laboratory, at a field size 100 mm × 100 mm and a focus–detector distance of 1 m was found to be

N_K = 1.10 Gy/div at 22.0° 101.33 kPa and 50 % rel. air humidity

Table 7

Recommended values of $(s_{w,air})$ as a function of depth (z) and mean energy at the phantom surface $\bar{E}_0$ for electron radiation. The values are taken from BERGER *et coll. (1975) with energy cut off Δ=15 keV, I(water)=71.3 eV and I(air)=9[illegible].9 eV*

Depth mm	$(s_{w,air})$ Mean energy at phantom surface $\bar{E}_0$/MeV 1	2	3	4	5	6	7	8
1	1.136	1.112	1.092	1.074	1.058	1.043	1.031	1.019
2	1.144	1.124	1.101	1.081	1.065	1.049	1.037	1.025
4	1.151	1.137	1.112	1.090	1.072	1.056	1.042	1.031
6	1.157	1.147	1.122	1.099	1.080	1.063	1.048	1.037
8		1.154	1.132	1.108	1.088	1.070	1.055	1.042
10		1.157	1.142	1.118	1.097	1.078	1.061	1.048
12			1.150	1.129	1.106	1.086	1.068	1.055
14			1.155	1.139	1.115	1.094	1.074	1.061
16			1.157	1.146	1.125	1.104	1.082	1.068
18				1.151	1.134	1.112	1.091	1.074
20				1.155	1.141	1.121	1.100	1.082
25				1.156	1.153	1.141	1.120	1.102
30					1.154	1.151	1.137	1.123
35						1.152	1.148	1.139
40								1.147
45								1.146

Depth mm	$(s_{w,air})$ Mean energy at phantom surface $\bar{E}_0$/MeV 9	10	12	14	16	18	20	22
0	1.011	1.00[illegible]	0.990	0.980	0.97[illegible]	0.965	0.958	0.952
5	1.023	1.015	1.001	0.990	0.981	0.974	0.969	0.962
10	1.036	1.027	1.012	1.000	0.990	0.982	0.975	0.968
15	1.050	1.038	1.023	1.009	0.998	0.989	0.982	0.974
20	1.066	1.053	1.033	1.018	1.006	0.997	0.988	0.980
25	1.083	1.069	1.045	1.0[illegible]7	1.013	1.004	0.995	0.986
30	1.103	1.087	1.058	1.037	1.0[illegible]	1.011	1.001	0.993
35	1.1[illegible]0	1.105	1.072	1.049	1.031	1.018	1.008	0.999
40	1.138	1.1[illegible]7	1.086	1.060	1.041	1.0[illegible]5	1.015	1.005
45	1.144	1.137	1.104	1.075	1.053	1.034	1.0[illegible]2	1.011
50		1.144	1.120	1.091	1.065	1.046	1.031	1.017
60			1.138	1.119	1.093	1.069	1.051	1.035
70				1.135	1.117	1.093	1.072	1.055
80					1.127	1.114	1.094	1.073
90						1.1[illegible]	1.118	1.097
100							1.1[illegible]8	1.118
110							1.1[illegible]	1.1[illegible]4
120							1.105	1.115

Depth mm	$(s_{w,air})$ Mean energy at phantom surface $\bar{E}_0$/MeV 25	30	35	40	45	50
0	0.944	0.931	0.923	0.917	0.912	0.9[illegible]
10	0.959	0.945	0.935	0.926	0.9[illegible]1	0.9[illegible]
20	0.971	0.956	0.944	0.934	0.9[illegible]	0.9[illegible]
30	0.981	0.965	0.952	0.942	0.934	0.9[illegible]
40	0.992	0.974	0.960	0.949	0.940	0.9[illegible]
50	1.003	0.983	0.968	0.956	0.946	0.9[illegible]
60	1.016	0.993	0.977	0.963	0.953	0.9[illegible]
70	1.030	1.004	0.986	0.971	0.960	0.9[illegible]
80	1.047	1.016	0.995	0.979	0.967	0.9[illegible]
90	1.065	1.030	1.006	0.988	0.9[illegible]4	0.9[illegible]
100	1.085	1.045	1.017	0.997	0.98[illegible]	0.9[illegible]
110	1.112	1.062	1.030	1.006	0.991	0.9[illegible]
120	1.122	1.079	1.043	1.017	0.999	0.9[illegible]
130	1.117	1.101	1.058	1.028	1.008	0.9[illegible]
140	1.105	1.113	1.073	1.041	1.018	1.0[illegible]
160	1.099	1.105	1.103	1.068	1.040	1.0[illegible]
180		1.094	1.101	1.094	1.064	1.0[illegible]
200			1.088	1.094	1.088	1.0[illegible]
220				1.081	1.088	1.0[illegible]
240				1.083	1.075	1.0[illegible]
260					1.072	1.0[illegible]
280						1.0[illegible]

The absorbed dose to air (inside the cavity) ionization chamber factor N_D recommended in the present protocol is

$$N_D = N_K k_{att} k_m (1-g)$$

$N_K = 1.10$ Gy/div; $k_{att} = 0.990$; $k_m = 0.991$; $g = 0.004$ — $N_D = 1.075$ Gy/div at 22 °C, 101.33 kPa and 50 [illegible] rel. air humidity

Measurements in an electron beam (1) A depth ionization curve is measured for a large beam si[illegible] at SSD=1.0 m in order to determine the mea[illegible] energy of the electrons at the phantom surface $\bar{E}_0$. In the measurements the effective point of meas[illegible]urement (p. 71) is used. The depth at which th[illegible] ionizations are reduced to 50 per cent (R_{50}) is deter[illegible]mined to 84 mm. From Fig. 3 the correspon[illegible] $\bar{E}_0$ value is obtained and $\bar{E}_0$=20 MeV.

(2) From the depth ionization curve a depth ab[illegible]sorbed dose curve is calculated using $(s_{w,air})$ fa[illegible] from Table 7. The depth of the maximum absorbe[illegible] dose was found to be 30 mm. The absorbed dose a[illegible]

this reference depth is then determined from a meas urement with the centre of the ionization chamber at this depth Insertion of the meter reading M (corrected for temperature pressure recombination etc) and p and $(s_{w,a,r})_u$ factors from Table 5 in eq (11) gives the absorbed dose in water at the refer ence point

$$\left.\begin{array}{l} D_{w,u}=N_D M\ p\ (s_{w,air}) \\ \quad N_D=1\ 075\ \text{Gy/div} \\ \quad p=0\ 990 \\ \quad (s_{w,a,r})_u=1\ 001 \end{array}\right\}\ D_w\ =1\ 065\ M_u\ \text{Gy}$$

Measurements in a photon beam (1) The photon beam quality was estimated from the ratio J_{100}/J_{00} which was 1 51 This corresponds roughly to a maxi mum photon energy of 17 MV according to Table 6 or Fig 4 The reference depth is then obtained from Table 3 as 100 mm The absorbed dose at this depth is measured with the centre of the ionization cham ber at this point If M_u is the meter reading (cor rected for temperature pressure recombination etc) eq (11) and Table 6 give

$$\left.\begin{array}{l} D_{w,u}=N_D M_u p_u (s_{w,air})_u \\ \quad N_D=1\ 075\ \text{Gy} \\ \quad p=0\ 985 \\ \quad (s_{w,air})=1\ 108 \end{array}\right\}\ D_w\ =1\ 173\ M\ \ \text{Gy}$$

(2) The absorbed dose at dose maximum is deter mined from the ratio of the depth ionization at 100 mm and dose maximum The depth ionization curve is used for evaluation of this ratio (The effective point of measurement 0 75 r should be used in measuring depth ionization curves)

Determination of absorbed dose at any point

Relative absorbed dose distributions should be related to the absorbed dose at the reference point which should therefore be included in all distribution determinations The distribution should apply to a large water phantom (page 68) A complete set of distributions should be available for all combina tions of energy field sizes SSD etc that are in use for radiation therapy The hospital physicist is re sponsible for all modifications of these distributions in clinical practice for instance the insertion of lead block and bolus

Beam axis absorbed dose distribution

Electron beams The beam axis depth absorbed dose distribution cannot be specified in a unique way from electron beam parameters such as ener gy field size and SSD The shape of the distribu tions is dependent on a large number of construc tional details of the accelerators and they are only partly contained in the beam parameters Therefore as a rule the distributions should be determined for each accelerator When accelerators of the same design are used (SVENSSON 1971) common beam axis depth dose distributions may be applied by de partments only after a check of some distributions by measurements Significant differences may ap pear as a result of individual adjustments and small differences in thickness of accelerator window foils and in collimator design etc

The relative depth absorbed dose distributions could be measured with ionization chambers semi conductor detectors liquid ionization chambers or ferrous sulphate dosemeters The choice of method depends on the instruments that are locally avail able The relative distributions should be checked against depth absorbed dose curves measured with an ionization chamber method or possibly when available ferrous sulphate dosemeters

The ionization chamber method is well estab lished for measurements at depths equal to or larger than that of the dose maximum In the measure ments of relative depth ionization curves the dis placement effect of the ionization chamber should be taken into account for cylindric chambers This effect should be corrected for by using an effective point of measurement This point has been deter mined by extrapolating the geometric displacement of the depth ionization curves measured by dif ferent sizes of cylindric chambers to a zero size chamber In the experimental determination the per turbation effect was not considered so this is auto matically corrected for in the use of the effective point of measurement The effective point of meas urement varies slightly with the electron energy $E_{p,0}$ and phantom depth and is 0 5 r to 0 75 r in front of the chamber centre where r is the radius (HETTINGER et coll 1967 DUTREIX & DUTREIX 1966 JOHANSSON et coll 1977) A value of 0 5 r is recom mended for electron radiation The recombination losses can be disregarded in the measurement of relative depth ionization curves for those chambers recommended in the present protocol for most treat ment units The relative depth ionization curves should be multiplied with $(s_{w,air})_u$ (Table 7) for dif ferent depth in order to convert these curves to rela tive depth absorbed dose curves

At small phantom depths the air ionization method might introduce uncertainties due to contamination of low energy electrons and an incomplete build up of δ ray spectrum If measurements at such depths are desired a liquid ionization chamber (HULTÉN & SVENSSON 1975) or thin ferrous sulphate dosemeters (SVENSSON & HETTINGER 1967) could be recommended Since in general it is only necessary to make these measurements in the calibration procedure after an accelerator installation joint measurements by different departments are recommended

It is often useful to investigate the therapeutic and physical properties of an electron beam and describe them by some simple parameter There are several reasons for introducing such parameters (BRAHME & SVENSSON 1976) thus the increasing number of electron accelerators of different types and often quite different beam qualities necessitates a simple unified comparison of the quality of one beam with another the desirability of simple and accurate beam diagnostics make it useful to focus attention on a few independent parameters characterizing the beam quality and finally from a therapeutic point of view it is important to define the treatment volume in a relevant and consistant way not least to simplify comparisons between different treatment centres

The parameters that are recommended for use appear in Fig 2 D_m the maximum absorbed dose along the beam axis D_s the surface absorbed dose at 0 5 mm depth (this depth has been chosen as it is accessible for accurate absorbed dose measurement and as it approximately corresponds to the radiation sensitive layer below the epidermis) D_x the photon background G the absorbed dose gradient R_{100} the depth of the absorbed dose maximum R_{85} the therapeutic range R_{50} the half value depth and R_p the practical range

Of particular importance for an electron beam are R_{85} and G Experimental and theoretic values for broad beams are given in Figs 6 and 7 A dose gradient below about 2 5 for large beams indicates that the scattering system and collimating system are of poor design and that unnecessary large volumes of normal tissue are irradiated in single beam technique

Photon beams The beam axis depth absorbed dose curves are less critically dependent on the beam parameters and simpler to measure than for electron radiation All the dosemeter systems mentioned for electrons can be used but should be checked against the air ionization method The difference between the relative depth absorbed do-

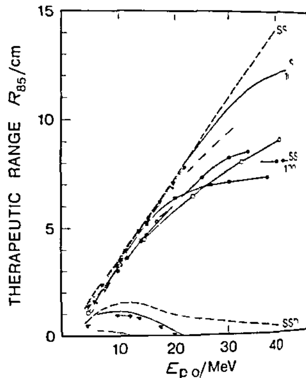


Fig 6 The therapeutic range R_{85} for large field sizes as a function of the most probable energy at the phantom surface E The SSD=1 m if not otherwise stated Experimental points are indicated with different symbols for different accelerators The upper two curves are theoretic data from BERGER & SELTZER (1969) the SSD=1 m curve is derived from inverse square law corrections Near surface lines are obtained for those beams with D_s/D_m<0 85

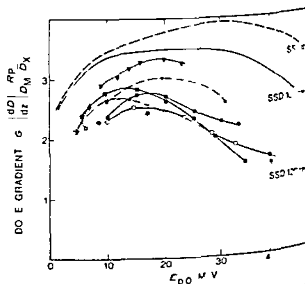


Fig 7 Dose gradients G for large field sizes as a function of the most probable energy at the phantom surface. The SSD= 1 m if not otherwise stated Experimental points are shown with different symbols for different accelerators The upper curves are theoretic data from BERGER & SELTZER (19.. SSD=1 m curve is derived from inverse square law corrections

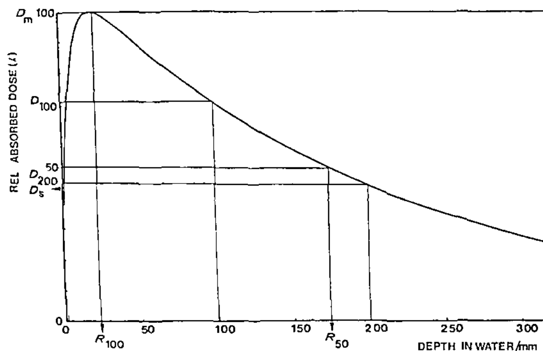


Fig 8 Beam axis depth absorbed dose distribution for photon beams Parameters often used to characterize the quality of the distribution are indicated $D_{100}/D_{200} \approx J_{100}/J_{200}$ which is input parameter for ($s_{w,i}$) in Table 6

and depth ionization curves when both are normalized at maximum may be 1 to 2 per cent at a large depth (SVENSSON 1971 NAHUM 1975) These differences can be disregarded for practical dosimetry In the measurement of depth ionization the displacement effect must be considered and the effective point of measurement must be used The effective point of measurement varies slightly with energy and phantom depth (HETTINGER et coll 1967 JOHANSSON et coll 1977) A value of 0 75 r is recommended for all energies and phantom depths The variations can be disregarded in practical dosimetry

Published depth dose data for different peak energies are available for different accelerators Those data should be checked by measurements of a few distributions both at small and large field sizes

Parameters describing the physical and therapeutic properties of the beam axis absorbed dose distributions are also useful for photon radiation D_s D_m R_{100} R_{50} are defined as for electron radiation (Fig 8) The ratio J_{100}/J_{200} is discussed on page 62 and is of importance for the choice of stopping power ratios

Iso-absorbed dose distributions

A semi-conductor detector connected to an automatic recorder may be used for the absorbed dose distribution determination in a water phantom for both photon and electron radiation beams This method is simple as the isodoses are directly plotted and corrections are often not necessary to carry out The spatial resolution is good as the sensitive layer of the detector is less than a few mm² Systematic errors could however be introduced for some accelerators especially for betatrons dependent on the radiation pulse shape and neutron contamination Furthermore the curves measured with the semi conductor may differ from the relative isodose curves at small phantom depths (BRAHME & SVENSSON 1976) The method should therefore be checked against ionization chamber measurements or possibly when available ferrous sulphate dose meters

The absorbed dose of electron beams at points outside the beam axis may be assessed by means of photographic film placed parallel to the beam axis in polystyrene phantoms A significant difference between the relative depth absorbed dose curve and the relative depth blackening curve exists at depths smaller than 20 mm (LOEVINGER et coll 1961 HETTINGER & SVENSSON 1967) The beam axis depth absorbed dose curve is first measured and is then used to assign a depth absorbed dose value to all points along the beam axis in the film Isodensity curves joining points in the film with the same net blackening are assigned to the depth absorbed dose

Table 8
Suggestion of a maintenance program for accelerators and ⁶⁰Co γ units

Check of		Frequency of check				
		Once a day	Once a week	Once a month	Once a quarter	Once a year
Light beam and pointers	p 74	^{60}Co acc				
Radiation beam and light beam agreement	p 74 Fig 5		^{60}Co acc			
All mechanical alignments	p 74 HPA (1970) AAPM (1975)					^{60}Co acc
Abs dose monitor and patient dose agreement	p 75	^{60}Co acc				
Abs dose monitor calibr factor constancy	p 75		acc	Co		
Abs dose monitor calibr factor (in)dependence of diff param	p 75			acc	acc	
Energy constancy	p 75		acc			
Radiation beam uniformity	p 75		acc			^{60}Co

at the point where they pass the beam axis It is frequently sufficient to construct the isodensity curves without correcting for background blackening

The same method for photon beams as for electron beams may be employed although the accuracy of this method in the penumbra region is less satisfactory The film method is often less accurate than measurements with semi conductor detectors Another approach is to use transversal measurements at 4 depths with an ionization chamber (decrement line method ORCHARD 1964 ORR et coll 1964) followed by computer calculation of the isodose curves (KALNÆS & MUNK 1972)

Maintenance program for the dosimetry of therapy units

The dosimetry data on which the irradiations are based shall be checked regularly for constancy The number of checks may depend to some extent on the behaviour of the particular therapy unit and its intended use If previous examinations indicate few and slow changes some decrease in their frequency may be satisfactory A maintenance program for ^{60}Co-γ units and accelerators is suggested in Table 8 where the frequencies are given on the assumption that dual absorbed dose monitoring (page 75) exists and that measurements of absorbed doses on patients are performed (page 76) All technical checking procedures prescribed by the manufacturer should be followed

The responsible physicist should for each therapy unit write instructions on how to carry out the relevant checks and their frequencies A logbook for recording these measurements shall be kept

Radiation beam alignment checks

A simple check on the light beam should be carried out daily A white card on which is drawn a square field is placed at the normal SSD With use of the numerical field size indicator a corresponding field size is set up and the light field is compared with the drawing Without moving the card this comparison is again performed after the radiation head is rotated through 180° It shall be checked that the cross hair light image and the front pointer indicate the centre of the light field and that the cross hair is projected on the back pointer tip

Checks of agreement between the light beam and the radiation beam should be performed every week and each time the light beam bulb is exchanged It is often convenient to combine this check with the check of the radiation beam uniformity The agreement should fulfil the values given in Geometric considerations (page 10)

Once a year a thorough alignment test should be performed Detailed information on relevant procedures has been given (HPA 1970 AAPM 19[illegible]

Absorbed dose monitor checks

Whenever performed the measurements of ab- ırbed doses on patients should be checked for greement with the absorbed dose monitor

On accelerators the constancy of the absorbed se monitor calibration should be checked weekly shall be possible to relate this check to the pri ary absorbed dose calibration of the monitor A ecial phantom made of plastic should be used be use of the convenience of handling The position the dosemeter should be close to the reference pth (Table 3) As several different reference pths might be of interest the phantom may con st of a basic block together with a series of slabs arked and used for this purpose only Monthly to uarterly a suitable series of such measurements ould be performed for testing the monitor preci on and stability and for examination of the calibra on factors for independence on monitor setting sorbed dose rate beam direction temperature d air pressure Also the dependence of the cali ation factor on wedges and field size flattening lter or scattering foils should be checked for con ancy

On ^{60}Co γ units the check of the absorbed dose onitor calibration may be less frequent but at least nce a month The check reveals timer errors hanges in shutter effects and possible changes in bsorbed dose rate caused by for instance redis ibution of the source (HANSEN 1972)

The ratio between the monitor reading and the etermined absorbed dose value shall not deviate by ore than ±2 per cent from the ratio determined at e original measurements

Radiation energy constancy checks

On therapy units which could have an uninten onal change in the selected radiation energy (i e etatrons linear accelerators) the energy constancy ould be checked once a week This is performed y checking the constancy of a ratio J_1/J_2 between nization measurements at two different depths he phantom for check measurements of absorbed ose monitor calibration should be used and J_1 is e measurement at the reference depth J_2 is meas red with an additional slab plastic material in front f the phantom For photon beams this slab should e approximately 10 cm thick and for electron eams about R_{50} minus the reference depth (Fig 2 able 3) The best geometric reproducibility is ob- ained if the source chamber distance is unaltered between measurements In some irradiation geometries this is not possible and the SSD should in stead be kept constant The ratios J_1/J_2 should be related to the relevant energy calibrations and the plastic slabs should be marked and used for this pur pose only

The ratios J_1/J should not deviate by more than ±1 per cent from the original ratios for photon beams For electron beams the deviation should be less than ±4 per cent

Radiation beam uniformity checks

The uniformity should be checked with the photographic film method (page 64) In each check the maximum blackening of the film and the blackening along the major axes and diagonals of the field should at least be determined A full evaluation of the film blackening is of great value as the uniform ity index then may be determined The check should be made weekly with cyclic permutation of some relevant irradiation conditions (e g radiation qual ity beam direction and field size) i e each com bination is checked at least every month The mini mum requirements given in the section Geometric considerations should be fulfilled in all uniformity measurements

Checks of absorbed dose given to the patient

The absorbed dose given to each patient shall be under proper control (ICRP 1970 LINDELL 1976 ICRU 1976) For this purpose dual absorbed dose monitoring systems and measurements of the absorbed dose on patients are recommended The dual monitoring system shall protect the patient against overdoses caused by equipment failures The pa tient dose measurements should detect any errone ous absorbed dose caused by equipment malfunc tions and human mistakes so that proper corrections in the following treatments can be performed

Absorbed dose monitoring systems

Malfunctioning of ^{60}Co-γ unit timers have been reported (VELKLEY 1975) Also mechanical mal functions in the beam control system (sticking shutter broken return spring etc) have caused ex cessive and unknown absorbed doses to patients It is recommended to provide ^{60}Co-γ units with two in dependent timer systems both capable to give a ter mination signal to the beam control system Detailed recommendations are given in Radiotherapy Ap-

paratus Safety Medical Panel (RASMP 1975) Dual timer systems coping with these recommendations are commercially available

Accelerators shall be provided with two independent absorbed dose monitoring systems which to advantage should have physically separated radiation detectors At least one detector system shall consist of a transmission chamber and measure the full beam at the patient side of any flattening filter or scattering foils Both monitors shall be capable of independently terminating the irradiation The integrated signals from the detectors should be digitally displayed on the console and at least one monitor should start from zero at the beginning of an irradiation The readings of at least one display instrument should be preserved in the event of any failure (including power failure) or interruption of the irradiation It should not be possible to start another treatment before the dose monitors and the presettings are reset The absorbed dose rate shall be indicated on the console and for each increment of the dose monitor readings an acoustic beat should be given If the dose rate exceeds a certain preset level (e g twice the normal dose rate) automatic termination of the irradiation should result From the radiation detectors signals should be derived which are proportional to the output in different parts of the radiation beam so that the beam uniformity can be detected An abnormal uniformity should result in an automatic termination of the irradiation For a given radiation quality the dependence of the dose monitor calibration factor on dose rate beam direction temperature and air pressure should be within 2 per cent The dependence on beam energy field size and flattening filter or scattering foil should be less than ±30 per cent

Absorbed dose measurements on patients

Absorbed dose measurements on patients could be divided into two levels of security For level one measurements are made at one of the first treatment occasions and every time a parameter (including patient anatomy) is changed This enables detection of systematic mistakes in the decided irradiation procedure Level two measurements are made at every treatment occasion enabling detection of occasional operator mistakes and equipment failures

Preferably level one measurements should be carried out in more than one point of the field A further improvement may be obtained in the reduction of the number of occasional operator mist[illegible] level one measurements are correlated to a [illegible] and confirm system Occasional changes in p[illegible] positioning and occasional equipment failures [illegible] however be revealed only by level two dosim[illegible] On older radiation units with only one dose m[illegible] system level two measurements may also repl[illegible] secondary dose monitor A thorough discuss[illegible] possible errors and control of the absorbed do[illegible] the patient for specific therapy procedures is [illegible] by MÖLLER et coll (1976)

Patient dosimetry systems to be used rout[illegible] should besides reasonable precision possess sim[illegible] ity of handling The demand for precision is d[illegible] mined by an action level for open beams of ±[illegible] cent For beams modified by a wedge or com[illegible] sating or blocking filter an action level of ±[illegible] cent should be used At least small conde[illegible] ionization chambers (Sievert chambers) the[illegible] luminescent dosemeters (TLD) and small s[illegible] conductor detectors with suitable (partial) bu[illegible] caps can fulfil these requirements

Appendix

For a proper understanding of the protocol [illegible] definitions are necessary taken from ICRU ([illegible] HPA (1970) and NACP (1972) or slightly modif[illegible]

Beam The electron or photon beam is the re[illegible] in space traversed by photons or electrons from [illegible] source Its edges are determined by the collim[illegible] its cross sections perpendicular to the beam ax[illegible] the field and its direction is that of photon or [illegible] tron travel

Beam axis Four types of beam axes can be [illegible] fined In a properly adjusted system all four [illegible] will coincide The definitions are

(1) Mechanical definition The *collimator r[illegible] axis* is defined as the rotational axis at the coll[illegible] head

(2) Geometric definition The *geometric b[illegible] axis* is defined as the line passing through the ce[illegible] of the beam flattening filter (or the main sca[illegible] foil or focus of scanning magnet system) an[illegible] centre of the final beam limiting diaphragms

(3) Radiation definition The *radiation beam* [illegible] (sometimes named reference axis) is defined as [illegible] line passing through the centre of the effective ra[illegible] tion source and the centre of gravity of the [illegible] within which the absorbed dose exceeds 50 per [illegible] of the maximum absorbed dose at the refer[illegible] plane in a phantom

(4) Light beam definition the *light beam axis* is fined as the line from the effective light source d the centre of gravity of the area within which the ht intensity exceeds 50 per cent of the maximum ht intensity at the phantom surface

Reference plane The reference plane is defined the orthogonal plane to the reference axis at a ven depth beneath and parallel to the phantom rface Recommended values for the depth are ven in Table 3

Reference point The reference point is defined the point of the inter section between the refer ce plane and the beam axis (reference axis)

Radiation field size The field size is defined in phantom at the depth of the reference plane with at plane at the proposed treatment distance The ld size is the area inside the 50 per cent level of e absorbed dose in the reference point The nu erical values of the field size are given as the stance between the 50 per cent level at the edges the major axes or the diameter of the 50 per nt level in a circular field

Light field size The light field size is defined at e surface of a phantom with the surface at the proposed treatment distance The light field size is the area inside the 50 per cent boundary of the light intensity the light intensity in the centre being 100 per cent The numerical values of the field size are given as the distance between the boundary of the major axes

Uniformity index The uniformity index is defined in the reference plane for a specified quantity (e g absorbed dose ionization net film density or cur rent from a semi conductor detector) as the ratio of the area containing points where this quantity ex ceeds 90 per cent of its value at the reference point and the area where it exceeds 50 per cent of the reference point value

Physical penumbra Physical penumbra is for a specified quantity as the lateral distance at the major axes between the 80 per cent and the 20 per cent of points of this quantity with the value at the reference point defined as 100 per cent

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ONCOLOGY

1980

MITOMYCIN C IN ADVANCED GALLBLADDER CARCINOMA

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and K JONSSON

Carcinoma of the gallbladder has an overall 5 year ırvival of about 5 per cent (WARREN et coll 1968 EVIN et coll 1976 PERPETUO et coll 1978) Prog osis is correlated mainly with the stage of invasion NEVIN et coll) and to some extent with the sex e and initial performance status (PERPETUO et oll) Tumor growth in the mucosa only (stage I) or 1 the mucosa and muscularis (stage II) had a 5 year urvival of about 65 per cent (NEVIN et coll) umor spread through the wall of the gallbladder to ıe serosa (stage III) or to the regional lymph nodes tage IV) had a 5 year survival of about 6 per cent NEVIN et coll) Patients with tumor spread beyond ıe regional lymph nodes (stage V) all died within ne year Gallbladder carcinoma treated with in avenous or intraarterial mitomycin C has shown bjective remission (SHIRAHA 1968 GUERRO et coll 972 MOERTEL 1973 SHIGENAGA 1976) Patients ith advanced gallbladder carcinoma have been eated with intravenous and intraarterial infusions f mitomycin C at this hospital and the results are ow reported

Material and Methods

Between 1977 and 1979 10 patients (8 women and men) with microscopically confirmed advanced allbladder carcinoma were treated Median age was 2 years (range 48–78) at the time of diagnosis Six atients had performance status I two patients had erformance status II and two patients had perfor nance status III (ZUBROD et coll 1960) Two pa ients had gallbladder carcinoma stage III and 8 stage V Cholecystectomy had been performed in 7 patients explorative laparotomy in 3 ileocecal re section in one regional lymph node extirpation in one and choledochotomy in one One patient was treated with melphalan and medroxy progesterone acetate before mitomycin C None of the patients had been irradiated All patients had a measurable tumor a leukocyte count above 4×10^9 cells/l and a platelet count above 125×10^9/l at the beginning of the mitomycin C treatment (Table 1)

Before each course Hb white blood cell count and platelet count were determined in all patients and the patients were examined physically Hepatic and renal function tests were carried out regularly as well as scintigraphy of the liver and ultrasound examinations repeatedly to evaluate the response The coeliac or the hepatic arteries were catheterized for the intraarterial infusions (Fig 1) Seven patients were given 2 to 5 courses of mitomycin C intraar terially with 10 to 24 days interval For infusion 10 mg mitomycin C was dissolved in 100 ml isotonic sodium chloride solution or 15 mg mitomycin C was dissolved in 150 ml isotonic sodium chloride solu tion The infusion was given at a rate of 6 ml/min After each infusion the catheter was withdrawn and the procedure was repeated for each intraarterial infusion After the intraarterial treatment 2 patients were given 5 fluoro uracil 1 000 mg/m² body surface intravenously in 6 hours on day 1 and day 2 and

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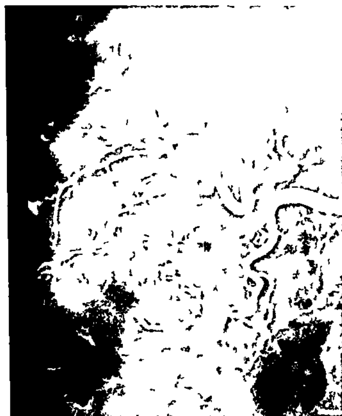


Fig 1 Hypervascular carcinoma of the gallbladder Case 10

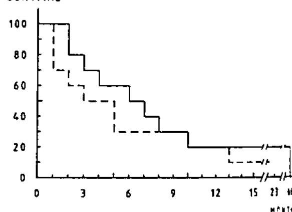


Fig 2 Survival (in per cent) from time of diagnosis (—) and beginning of mitomycin C treatment (---)

mitomycin C 6 mg/m² intravenously in 30 min on day 2 with 3 weeks intervals Two patients received a single intravenous infusion of mitomycin C

All other courses were given intravenously in a dosage of 10 mg/m with a median 3 weeks interval (range 2–11) Mitomycin C was dissolved in 100 ml isotonic sodium chloride solution and infused in to 30 min together with a fast running infusion of ml isotonic sodium chloride solution The dose was reduced according to myelosuppression (Table

The response was assessed according to the following criteria Stationary disease when the measurable tumor decreased or increased within per cent of the initial size for at least one month progressive disease when the tumor increased more than 50 per cent of the initial size or a new lesion appeared and treatment failure when patients died after one course of mitomycin C

Results

Stationary disease was obtained in 4 patients progressive disease occurred in 4 and treatment

Table 1

Data of the cases before mitomycin C treatment

Case No	Sex	Age	Performance status	Initial surgery	Stage of primary tumor
1	F	48	II	C+ICR	V
2	F	55	III	E	V
3	F	69	I	C	V
4	F	55	I	C	III
5	F	49	I	C+LNE	III
6	F	65	I	E	V
7	M	64	I	C+CD+GI	V
8	F	77	II	C	V
9	M	59	III	E	V
10	F	78	II	C+CDT	V

C=cholecystectomy E=explorative laparotomy ICR=ileocecal resection LNE=lymph node extirpation CDT=choledochotomy

Table 2

Scheme for dose modification of mitomycin C

Leukocytes (×10⁹ cells/l)	Platelets (×10⁹/l)	Per cent of planned dose
3.1–3.5	71–100	50
2.1–3.0		25
≤2.0	≤70	0

ilure in 2 patients. Eight patients with gallbladder ırcinoma stage V survived for a median 3 months ter mitomycin C treatment; 2 with stage III survived 13 and 23+ months, respectively (Table 3, ig. 2).

After the intraarterial infusions, one patient had a ocal hematoma in the groin, 5 patients had a mild ukopenia and one had a longlasting thromocytopenia. Otherwise, no severe side effects of ıe intraarterial treatment occurred.

After intravenous mitomycin C treatment 4 paients had a mild leukopenia for a short time and 4 atients had thrombocytopenia, extreme and onglasting in one of these. Nausea, diarrhea and xtensive alopecia occurred in one patient and seere gastrointestinal bleeding in one. No side effects rom other organ systems were noted. No drug reated deaths occurred.

Discussion

Mitomycin C has proved to be effective in several cases of gallbladder carcinoma (SHIRAHA; GUERRO et coll.; MOERTEL; SHIGENAGA). However, in the *present series no objective remission occurred in 10* cases of advanced gallbladder carcinoma. The result was statistically significantly lower (p=0.002, binomial test) than the 47 per cent objective remission rate in a compiled review of cases published by CROOKE & BRADNER (1976, 7 patients with objective remission of 15). The present result did not differ (p=0.31, binomial test) from another series of cases with an 11 per cent objective remission rate (3 cases of 27, SHIRAHA).

In 2 cases, myelosuppression was so severe that the mitomycin C treatment was discontinued. This corresponds to previous reports (CROOKE & BRADNER). No drug related deaths occurred. Thromboembolism was not encountered in any case, which is in accordance with previous experiences of intraarterial mitomycin C treatment at this hospital (MATTSSON et coll. 1977; HELLEKANT & SVANBERG 1978; HELLEKANT et col. 1978). This may be due to the duration of infusion; in long term intraarterial infusions thromboembolism was frequent (CLOUSE et coll. 1977).

Patients with advanced gallbladder carcinoma have an extremely poor prognosis. Radical surgery does not improve survival compared with cholecys

Table 3

Doses and results of mitomycin C in gallbladder carcinoma

Case No.	Mitomycin C treatment				Overall total dose (mg/m²)	Response	Survival (in months)	
	Intraarterially		Intravenously				After diagnosis	After mitomycin C
	No. of courses	Total dose (mg/m²)	No. of courses	Total dose (mg/m²)				
1	–	–	1	10	10	TF	7	1
2	–	–	1	10	10	TF	[illegible]	1
3	3	28	7	52	80	NC	10	10
4	3	32	5	33	65	NC	35+	23+
5	3	27	15	62	89	NC	66	13
6	3	30	–	–	30	PD	4	3
7	3	17	3	13	30	NC	6	5
8	–	–	6	53	53	PD	8	5
9	2	16	–	–	16	NC	2	1
10	5	41	–	–	41	PD	3	2

NC=stationary disease. PD=progressive disease. TF=treatment failure.

tectomy (DOWDY 1969) Single drug chemotherapy with 5 fluoro uracil has proved to be of no use (MOERTEL PERPETUO et coll) High voltage irradiation has given alleviation (MOERTEL SMORON 1977 KOPELSON et coll 1977) combination chemotherapy with 5 fluorouracil and nitrosourea or adriamycin has given objective remission (PERPETUO et coll) in small series of patients

The median survival in the present series was longer than in previous reports Thus 8 patients with carcinoma stage V survived for a median 5 months after diagnosis In other series of all stages of gallbladder carcinoma treated with or without chemotherapy median survival was 3 to 5 months (MOERTEL PERPETUO et coll) Two patients with carcinoma stage III who had a relapse after nonradical surgery survived longer after mitomycin C treatment (13 and 23 + months) than the patients with stage V (median survival 3 months) The prolonged survival may be due to less extensive or less malignant tumor or to a better effect of mitomycin C No objective remission occurred in the present series but the survival was prolonged compared with previous reports Therefore further trials with mitomycin C in gallbladder carcinoma are indicated

SUMMARY

Seven patients with advanced gallbladder carcinoma were given intraarterial infusions of 10 to 15 mg mitomycin C with 10 to 24 days interval 3 received intravenous infusion (10 mg/m²) with median 3 weeks interval Stationary disease was obtained in 4 patients progressive disease in 4 and treatment failures in 2 The median survival after mitomycin C treatment was 4 months (range 1–23+) Irreversible thrombocytopenia occurred in 2 patients and a severe gastrointestinal bleeding episode in one

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ROM THE DEPARTMENTS OF NUCLEAR MEDICINE AND OF ONCOLOGY AND RADIATION THERAPY THE RADIUM CENTRE AARHUS KOMMUNEHOSPITAL AND UNIVERSITY OF AARHUS DK 8000 AARHUS DENMARK

PENTAGASTRIN CALCIUM AND WHISKY STIMULATED SERUM CALCITONIN IN MEDULLARY CARCINOMA OF THE THYROID

K EMMERTSEN H F NIELSEN L MOSEKILDE and H HVID HANSEN

Medullary thyroid carcinoma (MCT) is a tumour of the calcitonin secreting parafollicular C cells of the thyroid gland The disease may be familial with an autosomal dominant mode of inheritance and often associated with other endocrine neoplasms

Determination of serum immunoreactive calcitonin (S iCT) concentrations is the most sensitive method for the diagnosis (TELENIUS BERG et coll 1975) but basal levels may be within normal range in cases of C cell hyperplasia and small carcinomas However calcitonin secretory capacity is abnormally high in these patients Determination of S iCT concentrations after stimulative procedures can therefore disclose the C cell neoplasm in an early stage (SIZEMORE & GO 1975 MILHAUD et coll 1975 TELENIUS BERG et coll 1977 HILLYARD et coll 1978 GRAZE et coll 1978)

Intravenous injection of pentagastrin is now the most commonly used stimulative procedure (TELENIUS BERG et coll 1977 GRAZE et coll) but superiority has been claimed for the use of short term calcium infusion (RUDE & SINGER 1977) or oral whisky (DYMLING et coll 1976 HILLYARD et coll) Therefore these three stimulative procedures for calcitonin secretion were compared in patients with proven MCT and in healthy controls

Material and Methods

The material comprised 6 patients (3 males and 3 females aged 30–65 years) all previously operated upon for MCT microscopically proven and with persistently elevated basal S iCT concentration and 8 healthy volunteers (4 males and 4 females aged 24–40 years) MCT was familial in one (Fig 3 E J) and sporadic in the other 5 patients

Serum concentrations of calcium phosphorus alkaline phosphatase and creatinine were within normal limits in all subjects Informed consent was obtained from all individuals

S iCT was measured in all subjects before and 2 5 15 and 30 min after stimulation with pentagastrin (Peptavlon 0 5 μg per kg body weight diluted in 1 to 2 ml isotonic sodium chloride given i v within 5 s) calcium (2 mg per kg body weight as calcium laevulatis 10% given intravenously during 1 min) and whisky (50 ml Johnny Walker Black Label by mouth) The order of the tests differed between the subjects During calcium infusion S iCT and serum calcium were measured simultaneously In each individual the calcitonin stimulation tests were initiated at 8 00 a m after fasting overnight The injections were given and the blood samples drawn through an intravenous catheter

S iCT was measured by radioimmunoassay as described previously (NIELSEN et coll 1979 NIELSEN & OLSEN 1979) using a commercial antibody to synthetic human calcitonin (Calbiochem U S A) and synthetic human monomer calcitonin (Ciba Switzerland) for standards and iodination The de

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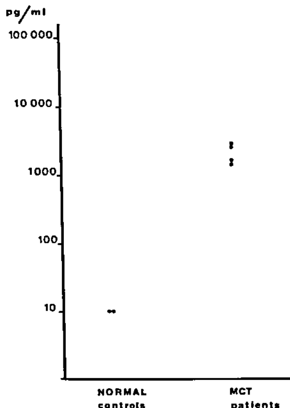


Fig 1

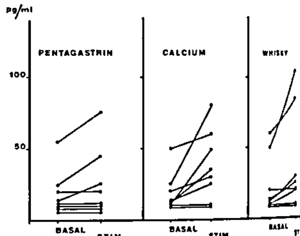


Fig 2

Fig 1 S iCT (pg/ml) in 6 patients with medullary carcinoma of the thyroid (MCT) and in normal controls

Fig 2 S iCT (pg/ml) in 8 healthy individuals before and after (maximum value) stimulation with pentagastrin 0 5 µg/kg body weight calcium 2 mg/kg body weight and whisky 50 ml

tection limit was 20 pg/ml Normal range was 0 to 120 pg/ml and detectable serum values could be found in 65 per cent of normal subjects The intraassay coefficient of variation was below 3 per cent at a serum concentration of 350 pg/ml and 14 per cent at a serum concentration of 30 pg/ml The interassay variation coefficient was 14 per cent at a S iCT concentration of 140 pg/ml

Statistical evaluation A significant increase in S iCT was defined as an increase exceeding twice the analytical error The relative increases in S iCT were compared using Student s t test for paired comparisons

Results

The basal S iCT values in MCT patients and normal controls are given in Fig 1 S iCT was increased in all patients with MCT ranging from 380 to 120 000 pg/ml S iCT was undetectable in 5 of the 8 volunteers and within normal limits (less than 120 pg/ml) in the rest

The maximum increases in S iCT during stimulation with pentagastrin calcium and whisky in the normal controls appear in Fig 2 A significant increase in S iCT ($p<0.05$) was found in 2 controls following pentagastrin in 3 following calcium and in 3 following whisky The maximum increase in S iCT was found 2 to 15 min following stimulation without differences between the stimulation procedures None of the stimulated values exceeded the upper normal basal range of 120 pg/ml The mean rise in serum calcium during calcium infusion was 1 4 mg/100 ml (range 1 0–1 7)

The effects of stimulation with pentagastrin calcium and whisky on S iCT in the 6 patients with MCT appear in Fig 3 A significant increase in S iCT ($p<0.05$) was found in all 6 patients following pentagastrin in 5 patients following calcium and in patients following whisky The maximum increases in S iCT were found 2 to 5 min following stimulation without differences between the stimulation procedures

The average absolute increase in S iCT was 81 5[illegible] pg/ml (range 2 150–400 000) following pentagastrin 23 400 pg/ml (range 0–115 000) following calcium and 13 800 pg/ml (range 0–80 000) following whisky The relative mean increase in S iCT was [illegible] per cent (range 130–1 475) after pentagastrin [illegible] per cent (range 0–686) after calcium and 38 per cent (range 0–89) after whisky The mean rise in serum calcium during calcium infusion was 1 3 mg/100 ml (range 0 4–1 9)

The greatest increase in S iCT occurred in 5 ,

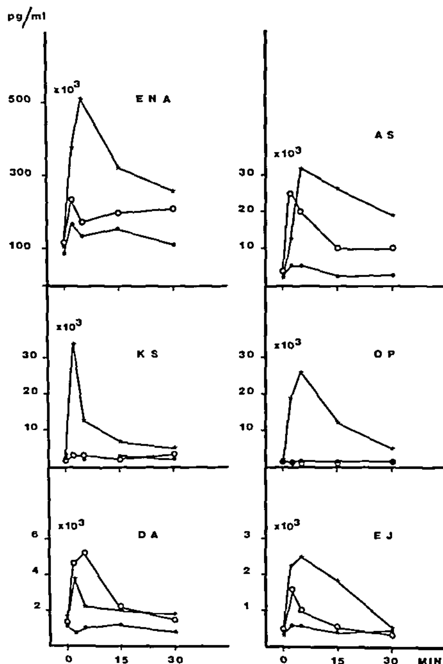


Fig 3 S iCT (pg/ml) in 6 patients with medullary carcinoma of the thyroid before and after stimulation with pentagastrin ( ) 0 5 μg/kg body weight calcium (O) 2 mg/kg body weight and whisky (●) 50 ml

tients after stimulation with pentagastrin and in one patient after stimulation with calcium No significant difference was found between the relative increase in S iCT following stimulation with pentagastrin or calcium However the relative increase in S iCT following whisky was significantly (p 0 02) lower than following pentagastrin where is no significant difference was found between the increases in S iCT following whisky and calcium

Discussion

The results showed that all 6 patients with MCT responded to pentagastrin with a marked increase in S iCT while 5 patients responded to short term calcium infusion and 3 to oral whisky The maximum stimulated S iCT values were found after pentagastrin in 5 patients and after calcium in one patient Among the 8 normal controls only a modest increase in S iCT after pentagastrin calcium and whisky was

found with no difference between the three procedures.

In several reports on screening for familial MCT (SIZEMORE & GO; TELENIUS-BERG et coll. 1977; GRAZE et coll.) it was stated that pentagastrin 0.5 µg/kg body weight given intravenously as a bolus is a potent stimulator for iCT secretion in patients with C-cell neoplasms, an excessive response being almost diagnostic. On the other hand RUDE & SINGER showed that short-term infusion of calcium 2 mg/kg body weight during one minute was a more potent stimulator for iCT secretion than pentagastrin in 4 patients with MCT. In this series one patient did not respond at all to pentagastrin.

A 4-hour infusion of calcium 15 mg/kg body weight was previously the standard stimulative procedure for disclosing the abnormally high S-iCT response in patients with C-cell neoplasms. However, this method is time consuming, frequently causes nausea and vomiting, and gives no advantage compared with pentagastrin (TELENIUS-BERG et coll. 1975; SIZEMORE & GO; GRAZE et coll.).

Injection of pentagastrin is followed by a sudden onset of generalized unpleasantness with oppression and abdominal discomfort lasting for about 2 min. Short-term calcium infusion produces less severe side effects, but may give nausea and a general feeling of warmth.

As a convenient and well tolerated stimulative procedure oral whisky has been introduced. Ingestion of 50 ml whisky increased S-iCT by a mean of 185 per cent in 14 patients with MCT (DYMLING et coll.). This method was subsequently used for detection of familial MCT (DYMLING et coll.; HILLYARD et coll.). In these series patients with basal S-iCT concentrations within normal range and abnormally high S-iCT responses to whisky did not undergo thyroidectomy with microscopic examination for C-cell neoplasms. Thus the frequency of true and false positive reactions could not be determined. Likewise, when evaluating stimulative procedures *in family members* at risk with borderline elevation of S-iCT, the frequency of false negative reactions cannot be evaluated unless all family members undergo thyroidectomy. Therefore the three stimulative procedures were tested in patients with known C-cell neoplasms, assuming that results obtained in these patients are valid also in patients with small C-cell neoplasms and borderline elevated S-iCT concentration. Using the iCT assay, whisky was a weak and unreliable stimulator for S-iCT compared with pentagastrin in 6 patients previ[ously] operated upon for MCT and with elevated b[asal] S-iCT concentrations indicating persistent C-[cell] neoplasia. Similar results have been obtained b[y] TELENIUS-BERG et coll. (1977).

In screening for familial MCT intravenous pen[ta]gastrin is recommended as the stimulative a[gent] with measurement of S-iCT concentrations befo[re] and 2 and 5 min after injection. Considering the single patient with MCT of RUDE & SINGER wh[o] did not respond to pentagastrin, short-term calc[ium] infusion should be carried out if results obtai[ned] after pentagastrin are equivocal or if clinical susp[icion] persists despite a negative S-iCT response to pentagastrin.

SUMMARY

The efficiency of pentagastrin, calcium and whisky in raising serum immunoreactive calcitonin (S-iCT) concen[]trations was analysed in 6 patients with medullary car[]cinoma of the thyroid and in 8 healthy controls. All patients responded to pentagastrin with a significant in[]crease in S-iCT, 5 responded to calcium and only 1 to whisky. In the 8 controls no or only a modest increase in S-iCT occurred following pentagastrin, calcium and whis[]ky with no difference between the three. It is concluded that pentagastrin is the most useful stimulative agent for iCT secretion in patients with C-cell neoplasms. In selected cases the additional use of calcium could be ad[]vantageous.

ACKNOWLEDGEMENTS

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NEUTRON RADIATION THERAPY OF PAROTID GLAND TUMORS

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The first report on neutron treatment of tumors ising from the parotid gland appeared in 1942 (STONE & LARKIN) By 1948 3 of 9 patients treated ith neutrons were still alive but all had distressing te effects (STONE 1948) All 3 survivors had troue with jaw movement skin atrophy absence of ibcutaneous fat and subcutaneous induration One ad an ulcer during the third year after treatment hich healed One developed radiation osteitis and raining of the sinus during the sixth year after eatment Since STONE recognized that the quality f survival is as important as the length of survival e discouraged further use of neutrons for treating arotid tumors as well as other malignant tumors

Following STONE s clinical trial FOWLER & IORGAN (1963) showed that the relative biologic ffectiveness (RBE) of neutrons relative to photons icreases with decreasing neutron dose per fraction his led to the realization that STONE may have ised excessively high doses by relying on RBE neasured in experimental animals at large neutron loses to determine the dose equivalency of neutrons nd photons at lower clinical neutron doses per frac ion This resulted in renewed interest in neutron herapy and preliminary clinical investigations were indertaken in England in the late sixties and later on n the United States

HENRY et coll (1978) and CATTERALL (1980) re ently have published their results for neutron herapy of salivary gland tumors CATTERALL found hat 30 of 34 patients had complete tumor regres ion In contrast to the results of STONE CAT ERALL reported only 7 complication 4 of which completely recovered HENRY et coll reported that neutron therapy resulted in 100 per cent local con trol rate for tumors less than 3 cm 75 per cent for those between 3 and 6 cm and no local control rate for tumors larger than 6 cm in diameter Similar to the results of CATTERALL HENRY et coll reported no long term complications at the time their results were presented

Unfortunately in none of these reports sufficient information is given with respect to individual treatment protocol tumor site (i e parotid submaxillary etc) tumor dose tumor size regional node involvement follow up time or survival time to make a meaningful comparison with the neutron treated patients of STONE or with patients treated with conventional photons or surgery The late ef fects noted by STONE were based on patients with parotid tumors who had been followed up for 60 75 and 92 months Therefore a critical question is how many patients in the current neutron trials have had comparable follow up times to develop similar late effects In this regard a special opportunity exists at the University of Washington where 9 patients with parotid gland tumors have been treated with neu trons It has been possible to compare these neutron results with those of STONE as well as with published results involving photon irradiation and surgi cal treatment of parotid tumors Furthermore in the Seattle area a central tumor registry exists referred to as the Cancer Surveillance System at the Fred Hutchinson Cancer Research Center A particularly

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Table 1

Extent of disease codes for the parotid gland

	Local vessel invasion	Regional lymph nodes	Size of primary tumor			
			2 cm or less	2 1 to 3 9	4 cm or more	Size unknown
Primary tumor no direct extension no distant involvement						
Single focus and						
entirely within benign tumor capsule	No	No	10	15	20	25
substance of parotid gland invaded	No	No	11	16	1	[illegible]
Confined to substance of parotid gland but multicentric foci	No	No	12	17	22	[illegible]
Localized no detailed information	No	No	14	19	24	[illegible]
Single focus and						
entirely within benign tumor capsule	Yes	No	30	35	40	45
substance of parotid gland invaded	Yes	No	31	35	41	46
Confined to substance of parotid gland but multicentric foci	Yes	No	32	37	4	[illegible]
Localized no detailed information	Yes	No	34	39	44	49
Single focus and						
entirely within benign tumor capsule		Yes	50	55	60	65
substance of parotid gland invaded		Yes	51	56	61	66
Confined to substance of parotid gland but multicentric foci		Yes	52	57	62	6
No detailed information of above		Yes	54	59	64	69

Regional lymph nodes for this site (first chain of drainage) parotid gland group

useful feature of this tumor registry is that for most patients with malignant tumors the extent of the disease is expressed in a two-digit code (Tables 1 2) Since the coding is done by experienced tumor registrars at each participating hospital a third party evaluation of the extent of disease for each patient is available This information also allows selection of control patients with the same severity of the disease as the neutron treated patients but who were treated with photons As a result it has been possible to make a preliminary evaluation of the efficacy of neutrons in treating parotid gland tumors based on a retrospective analysis of the clinical data for photon and neutron treated patients

Method

The 9 patients with parotid gland tumors were treated with neutrons between May 1974 and January 1977 The patient charts were reviewed to obtain the treatment protocol (radiation alone or surgery plus radiation) type of irradiation [illegible] or combined neutron/photon) tumor size [illegible] size tumor type nature of disease (primary or [illegible] current) follow up time survival time and com[illegible] cations The extent of disease code was available [illegible] 6 cases from the Cancer Surveillance Sys[illegible] try The other 3 cases were retrospectively [illegible] based on the information in the patients re[illegible] Photon treated patients in the Cancer Surve[illegible] System who had the same type of tumor and [illegible] extent of disease were selected as controls D[illegible] for neutron treated patients were also retro[illegible] ly staged using the staging system described by [illegible] et coll (1977)

Patients were treated with neutrons prod[illegible] bombardment of the beryllium target w[illegible] deuterons at the University of Washington [illegible] tron Depth dose and skin sparing properties of [illegible] neutron beam are similar to those for [illegible]Cs [illegible] rays Physical doses were measured [illegible] equivalent ionization chambers Patients re[illegible]

Table 2

Extent of disease codes for the parotid gland

	Involvement of regional lymph node	
	No	Yes
Limited direct extension		
Penetration of capsule of gland into connective tissue	70	80
Involvement of		
neural sheath (facial nerve)	71	81
muscle	72	82
skin	73	83
periosteum of mandible	75	85
pharyngeal mucosa	76	86
more than one (70–76) or (80–86)	79	89
Further direct extension		
Ulceration of skin	90	−0
Mandible	91	−1
Skull	92	−2
External auditory meatus	93	−3
Major blood vessels	95	−5
More than one (90–95) or (−0 through −5)	99	−9
Distant involvement		
Distant site	&1	&6
Distant lymph node	&2	&7
Distant site and distant lymph node	&3	&8

Regional lymph nodes for this site (first chain of drainage) parotid gland group

only neutron treatment were treated twice (Monday and Friday) or 4 times a week (Monday, Tuesday, Thursday and Friday). Patients receiving combined (neutron/photon) irradiation were treated twice a week with neutrons (Monday and Friday) and 3 times a week with photons (Tuesday, Wednesday and Thursday). The total irradiation times were between 6 and 8 weeks. The total dose was calculated by multiplying the neutron dose by the RBE of 3.0 which was used clinically (GRIFFIN et coll. 1980) and adding that figure to the dose delivered by photons.

Survival rates were determined by the life table method of MACDONALD (1962).

Results

The profile of the 9 neutron treated patients with respect to the severity of disease and treatment characteristics is presented in Table 3. Seven of the patients had primary tumors and 6 of these had either most or all of the parotid tumor resected before the neutron irradiation. The 3 patients receiving neutron therapy alone had persistent or recurring tumors following treatment. These 3 patients also had the largest tumors in the series and 2 had regional node involvement. The tumor type in the majority of the patients was either adenoid cystic carcinoma or mucoepidermoid carcinoma. Seven of the patients are now deceased, but one, who had the least severe disease, has a survival time of 5 years.

The profile of 6 photon treated patients obtained from the Cancer Surveillance System registry, who had equal or greater severity of disease in comparison to the neutron treated patients as indicated by the extent of disease code, is presented in Table 4. Of these 6 patients 3 had regional node involvement and 5 had adenocarcinoma. Five patients had been operated upon before irradiation. Three of these patients are deceased.

The normal tissue complications noted in the patients' records for neutron treated patients surviving

Table 3

Neutron treated parotid gland tumors

Case No	Disease	Tumor size (cm)[a]	Node size (cm)[a]	Tumor type	EOD[b]	Stage[c]	Treatment[d]	Dose[e]	Tumor status[f]	Vital status	Fu w time (mo)[g]
1	Primary	?	0	Adenoid cystic carcinoma	31	I	Surgery+ mxRT	67 0	NED	Alive	60
2	Primary	4	0	Mucoepidermoid carcinoma	99	III	Surgery+ mxRT	58 5	M	Dead	10
3	Recurrent	15	0	Mucoepidermoid carcinoma	&1	IV	nRT	55 5	P+M	Dead	9
4	Primary	4	0	Adenocarcinoma	91	III	Surgery+ mxRT	63 7	M	Dead	?8
5	Recurrent	?	3	Acinic cell carcinoma	−9	IV	Surgery+ nRT	60 0	R	Dead	40
6	Primary	9 5	0	Adenoid cystic carcinoma	71	III	nRT	60 0	R	Dead	13
7	Primary	4	2	Squamous cell carcinoma	&3	IV	Surgery+ nRT	56 0	M	Dead	1?
8	Primary	8	0	Undifferentiated carcinoma	99	III	nRT	64 5	P	Dead	13
9	Primary	4	0	Adenoid cystic carcinoma	71	III	Surgery+ mxRT	64 8	NED	Alive	8

Tumor size measured at the largest dimension

Extent of disease (EOD) codes were obtained from the Cancer Surveillance System survey and are defined in Tables 1 and Ca 6 and 8 were uncoded and therefore were retrospectively coded based on the information in the patient records

Diseases are retrospectively staged based on the staging system used by Fu et coll

[d] mxRT indicates mixed neutron and photon irradiation nRT indicates neutron treatment

Doses reported are in Gy and were calculated by multiplying the neutron dose by the RBE of 3 which was used clinically (Gr coll) and adding the dose delivered by photons

[f] Tumor status at last follow up NED — No evidence of disease R — Recurrent local disease P — Persistent local disease M Metastatic diseases

Follow up times are measured from the date of diagnosis The follow up times of deceased patients are the actual survival times

Table 4

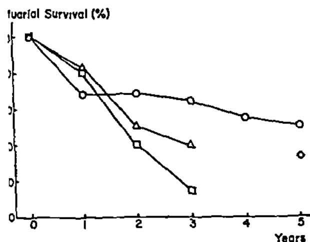
Photon treated parotid gland tumors

Case No	Tumor type	EOD[a]	Treatment	Vital status	Follow up time (mo)[b]
10	Adenocarcinoma	&1	RT	Dead	18
11	Mucoepidermoid carcinoma	81	Surgery+RT	Alive	58
12	Adenocarcinoma	&1	Surgery+RT	Dead	23
13	Adenocarcinoma	81	Surgery+RT	Alive	?6
14	Adenocarcinoma	89	Surgery+RT	Dead	8
15	Adenocarcinoma	7?	Surgery+RT	Alive	25

All cases were obtained from the Cancer Surveillance System

Extent of disease (EOD) codes were obtained from the Cancer Surveillance System survey and are defined in Tables 1 and 2

Follow up times are measured from date of diagnosis The follow up times of deceased patients are the actual survival time



tuarial survival of neutron and conventionally treated patients th advanced carcinoma of the parotid gland Error bars are nitted for clarity O Conventional therapy stage III FU et ll (? patients) Δ Conventional therapy all cases from incer Surveillan e System (present report 6 patients Table 4) STONE (9 patients) □ Neutron therapy primary tumors age III (present report 5 patients Table 3) The 3 year survival tes ± SD are 63 3±10 2 40 0±21 9 ≥33 3±11 1 and 11 1± 8 respectively

Table 5

N rm il tissue complicati ns f r neutron treated patients ith a foll up time of at le ist 2 years

Case No	Complications	Approximate time of occurrence (mo)
1	Significant fibrosis telangiectasia and 5 mm ul eration in the treated area	58
	Headaches intermittent right body seizures with aphasia and left eye pain	60
4	None	
5	Neck with very marked areas of necrosis	30
	Several small ulcerations in treated area	33
9	Local pain dryness of mouth and tissue thickening in treated area	25

Case number refers to cases in Table 3
Complications are those expressed in the patients records
Times measured are from date of diagnosis

nger than 2 years are presented in Table 5 In 3 of 4 ases evidence of significant alterations in normal ssues (primarily the skin) within the treatment vol me was found Of particular interest is Case No 1 who had a parotid tumor on the left side and has the ngest survival time to date Significant fibrosis langiectasia and a 5 mm ulceration developed in e treated area 58 months after treatment At 60 onths after the irradiation this patient developed eadache intermittent right body seizures with phasia and left eye pain

The actuarial survival curve for the 5 neutron eated primary tumors stage III (Cases 2 4 6 8 9 able 2) appears in the Figure For comparison the urvival curve for 22 patients stage III treated with onventional therapy (surgery photons or a com ination thereof) reported by FU et coll as well as a urve for all 6 photon treated patients obtained from e Cancer Surveillance System (Table 3) are also lotted The results show that the survival rate one ear after treatment for the 5 neutron treated pa ents is similar to those who received conventional eatment However at longer times after treatment onventionally treated patients had a better survival ate than the neutron treated patients In addition e long term survival of the neutron tre ited patients not better than for the incurable p itients treated y STONE in the 1940's

Discussion

HENRY et coll and CATTERALL used local control as a basis for conclusion of results In the present series this basis is patient survival STONE & LARKIN in their initial clinical neutron trials recognized the importance of survival as a basis for conclusion when they stated the ultimate aim of all treatment is the survival of the patient and the result of (neu tron author s incertion) treatment must be judged by this criterion However in considering the results of the present series in terms of survival it is impor tant to recognize that this is a preliminary evaluation of the efficacy of neutron therapy of parotid gland tumors since it is based on a small number of pa tients in a non randomized trial to conclusively prove or disprove the superiority of neutrons ran domized clinical trials involving more patients are needed Nevertheless it is of value to estimate the trend expected in this renewed treatment as soon as possible and in particular to compare the early neu tron results of STONE with these more recent efforts

Unfortunately the clinical results in the present series indicate no improvement in survival rate for neutron treated primary parotid gland tumors stage III as compared with conventional treatment (Fig ure) This is in spite of the fact that none of the 5 neutron treated patients had regional node involve ment whereas one half of the patients in the Cancer

Surveillance System (Table 4) and one third in the series of FU et coll did have regional node involvement Furthermore a possible favorable bias towards neutrons in this comparison is indicated in that the prognosis with regard to survival for the tumor types (i e adenoid cystic and mucoepidermoid carcinoma) in most of the neutron treated patients on the whole is better than that for adenocarcinoma (FU et coll) the tumor type in 5 of 6 photon treated patients (Table 4) It is also of interest that with a maximum follow up time of less than 5 years only 2 of the present 9 patients treated with neutrons are presently alive (Table 3) This result is not better than that of STONE who reported 3 of 9 patients surviving longer than 5 years

An argument frequently expressed for the poor survival results obtained is that the disease has been so extensive in the neutron treated patients that it is unlikely that any therapeutic method could control the tumors Therefore the biologic advantages of fast neutrons will only be evident by treating less extensive malignant tumors (PARKER et coll 1977 CATTERALL et coll 1977) Presumably this would mean carcinoma stage II of the parotid gland First of all it is noteworthy that in 1942 when only the early effects were evident STONE & LARKIN expressed a similar sentiment when they stated there fore it should now be permissible to treat small cancer lesions that are more likely to be cured than the extensive ones However more important patients with malignant tumors of the parotid gland stage II have a 5 year survival rate of approximately 75 per cent with conventional therapeutic methods (FU et coll) Therefore to detect a 15 per cent increase in survival after neutron treatment (i e from 75 to 90%) at a significant level of 5 per cent with a probability of detection of 80 per cent would require 76 patients being treated with conventional methods and the same number of patients treated with neutrons (COCHRAN & COX 1957) The scarcity of parotid gland tumors stage II would seem to preclude such a trial

Of particular importance in the present series is the observation that for 3 of the 4 patients surviving longer than 2 years considerable tissue damage occurred in the treatment volume (Table 5) This is to be expected since WITHERS et coll (1978) have shown that the neutron RBE for late skin damage in pigs (considered a good animal model for human skin) is 3 1 to 3 4 for a 50 MeV deuteron produced neutron beam Correcting this RBE for the 20 per cent greater biologic effect of the Univers[illegible] Washington 22 MeV deuteron produced ne[illegible] beam (HALL 1977) results in a RBE for late d[illegible] damage of 3 7 to 4 1 Since the RBE of 3 0 was [illegible] clinically for calculating the equivalence of [illegible] and photon doses this would mean that the p[illegible] received approximately a 10 to 36 per cent over[illegible] posure to skin Since the dose response curve f[illegible] late complications in the head and neck region [illegible] humans is steep (SHUKOVSKY 1970 HERRING 19[illegible] such an increase in radiation dose should sub[illegible] tially increase the late normal tissue complica[illegible] rate Therefore lower tumor doses may be nec[illegible] sary in future neutron clinical trials to avoid t[illegible] complications However it should be recog[illegible] that in reducing the doses the probability of t[illegible] cure with neutrons is also reduced

Finally the higher local control rates reported [illegible] CATTERALL for salivary gland tumors treated [illegible] neutrons have resulted in these tumors being sing[illegible] out as being encouraging for neutron the[illegible] (FIELD 1976 FOWLER 1976 DUNCAN 1978) A[illegible] when only the early effects were evident STONE [illegible] LARKIN expressed a similar feeling when they s[illegible] the effects of neutrons on tumors have been s[illegible] as to encourage further study in selective case[illegible] Surely the parotid gland tumor was one of the c[illegible] referred to by STONE & LARKIN since at that t[illegible] the 3 parotid gland tumors treated were obser[illegible] to respond unusually well to neutron therapy [illegible] all 3 cases the tumors disappeared com[illegible] after treatment (STONE & LARKIN) Therefore [illegible] remains to be seen if the high local control r[illegible] reported for neutron treatment of salivary g[illegible] tumors in current clinical trials (HENRY et c[illegible] CATTERALL) is translatable to higher survival r[illegible] with no concomitant increase in late normal t[illegible] complications

SUMMARY

A retrospective analysis of 9 patients treated w[illegible] lotron fast neutrons for tumors arising from th[illegible] gland is presented In 3 of 4 patients who survived lo[illegible] than 2 years significant normal tissue damage o[illegible] within the treatment volume Five patients tre[illegible] neutrons or surgery plus neutrons for primary [illegible] stage III did not have a better prognosis wi[illegible] survival than a comparable group of patients trea[illegible] conventional methods (i e surgery ph[illegible] or a [illegible] bination thereof) These results appear to be [illegible] those obtained for parotid tumors treated with ne[illegible] the early 1940 s by STONE

ACKNOWLEDGEMENT

This investigation was supported by Grant No CA ...31 awarded by the National Cancer Institute DHEW ...e author is grateful to Dr K L Jackson for his helpful ...gestions and to P D Thrower M Marino and M G ...ga for their technical assistance The assistance of the ...ffs of the Medical Records Department of the Universi... Hospital and the Cancer Surveillance System of Fred ...itchinson Cancer Research Center is also acknowl...ged

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EFFECT OF DIFFERENT RADIATION FRACTIONATION SCHEDULES ON METASTASES FROM AN OESOPHAGEAL CARCINOMA

C MERCKE I L LAMM P NILSSON T LANDBERG
C H HÅKANSSON and E HAMMAR

The results of radiation therapy of a malignant umour depend on several factors the most impor ant in clinical routine being the type and size of he tumour the proper delineation of the target volume the size of the total target absorbed dose and its fractionation and the application of chemical or physical agents that may modify the radiation esponse

Most biologic information is derived from in vitro ell systems and experimental animal tumours These systems cannot be said to reflect the complex n vivo conditions of spontaneously appearing tu nours in man The present investigation is an at tempt to characterize some inherent biologic prop rties of a human tumour in vivo from the results of irradiation of metastases from an oesophageal carcinoma using different fractionation schedules

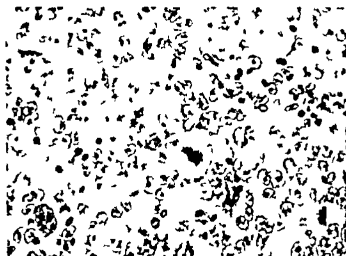
Material and Methods

A 70-year old woman with a previous history only of psoriasis developed swallowing disturbances At radiography a bulging tumour was demonstrated in the oesophagus measuring 8 cm × 4 cm ×4 cm on the films Biopsy (Fig 1a b) showed an anaplastic probably epithelial malignant tumour without de finite glandular or squamous differentiation No features of malignant lymphoma or melanoma were found Assuming the tumour to have the form of a cigar shaped rotation ellipsoid its volume was cal culated to be 65 cm³ The patient was considered inoperable and external irradiation was given using a 6 MV linear accelerator One anterior and one posterior field (16 cm × 8 cm) were irradiated with beam directions 0° and 180° respectively One frac tion was administered daily for 5 days a week and a total absorbed dose of 40 Gy was given in 20 frac tions over 29 days the dose specification being in agreement with the recommendations in the ICRU Report 29 (1978) The tumour regressed rapidly and was no longer demonstrable 6 and 12 weeks after conclusion of treatment Four months after the last irradiation day a local recurrence was demonstrated at radiography

When the oesophageal tumour was still in com plete remission the patient developed multiple sub-cutaneous tumours all being of the same micro-scopic type as the oesophageal tumour as con firmed by means of fine needle aspiration biopsy (Fig 1c d)

It was decided to irradiate the metastases Since the oesophageal tumour had responded well initi ally the fractionation schedule used i e 40 Gy in 20 fractions with treatment 5 days a week was taken as a reference Then other schedules were chosen giving the same radiation effect on normal connec tive tissue in terms of Cumulative Radiation Effect (CRE=13 4 KIRK et coll 1971) An attempt was made to analyse the corresponding responses with

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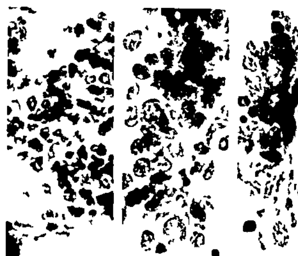


Fig 1 a) Biopsy from oesophagus undifferentiated malignant tumour with cellular pleomorphism and many mitoses H & E ×360 b) Imprint preparation from the same specimen H & E ×360 c) and d) Fine needle biopsies from two metastases in left upper arm Cell clusters with some vague epithelial formations MGG ×360

respect to biologic effect even if the parameters of the schedules selected were restricted by the clinical situation Five different fractionation schedules were used and altogether 7 metastatic tumours were irradiated (Fig 3) These were also given with low LET radiation with the same RBE value 11 MeV electrons from a Betatron or ^{60}Co gamma rays The field sizes were chosen to encompass the palpable tumours with a margin of at least 2 cm Chemotherapy was not administered Each field irradiated with electrons was evaluated for any skin reaction at each fraction and during the subsequent follow up The reactions were divided into two groups erythema (=1) and dry desquamation (=2)

As the metastases were subcutaneous their sizes and shapes were easily evaluable by means of palpation and all tumours were well demarcated The skin was freely movable and the distance from the tumours to the skin surface was measured to be 3 mm All the tumours had the form of rotation ellipsoids one being disc shaped and the others cigar shaped The volume of a rotation ellipsoid is given by

$$V=\frac{\pi}{6} d_1 d_2 d$$

where d_1 and d are the two independent axes of the ellipsoid (the longest and shortest diameters) and d_2 is the smaller axis for a cigar shape while it is the larger axis for a disc schape At each irradiation and on several occasions after treatment the two axes d_1 and d were measured with callipers independently by three examiners and the tumour volume calculated according to the formula mentioned

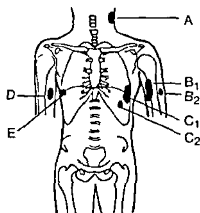
The location and initial size of the different tumours as well as data on the absorbed dose and fractionation are given in Fig 2

One additional tumour was not irradiated but followed for 57 days and then extirpated The tumour had the same morphology as the other tumours

Results and Discussion

In Fig 3 the increase in accumulated absorbed dose with time is given for the 5 fractionation schedules together with different kinds of skin reactions both when first noticed and when most marked After conclusion of treatment all observed skin reactions subsided Skin reactions occurred only with the two schedules having the largest number of fractions whereas no reaction at all occurred with those having few fractions even though the CRE value was the same for all the schedules However as TURESSON & NOTTER (1975) stated equal CRE values during the radiation treatment are not simultaneously related to identical skin reactions as these are delayed It is therefore difficult to compare reactions during the period of treatment itself and to assess the results at the last occasion of treatment

The tumour followed for 57 days progressed steadily before surgical excision and increased in size by approximately a factor of 11 (from 0



...ur ...ol	Volume (cm³)	Absorbed dose (Gy)		No of fractions	No of days
		Accumulated	At each fraction		
	3 68	40	2 00	20	30
	8 66 / 0 64	30	3 34	9	11
	4 63 / 1 03	29	5 80	5	30
	1 64	17 80	8 90	2	3
	0 65	35 40	2 95	12	30

Site and volume at start of treatment of the different sub ...neous tumours and data on target absorbed dose and frac ...ation Tumours B and B were treated with ⁶⁰Co the other ...ours with electrons (11 MeV) from a Betatron

... to 4 18 cm³) From the two observations made tumour volume doubling time T_d may be cal ...ated assuming exponential tumour growth to ±5) days

The size of the different tumours as measured ...ing and after the treatment period appears in ... 4 The uncertainties in all the measurements the linear dimensions of the metastatic tumours ...re estimated to ±1 5 mm This gives the follow... approximate uncertainties in the volume calcula...ns taken at three representative levels (5 0±1 0) ...³ (0 50±0 25) cm³ and (0 050±0 045) cm³

All metastases regressed completely and did not ...cur during a follow up time of 8 months There...re it is not possible to state if the absorbed dose ...as in excess of the necessary one to achieve cure ...n the other hand the primary oesophageal tumour ...curred after 4 months

It appears from Fig 4 that no decrease of the ...mours occurred during the first week of irradia...on rather an increase in particular when large ...oses were given at each fraction It is reasonable ...o attribute this increase to an initial oedema even if killed tumour cells i e cells incapable of divid ing may still add to the tumour volume Moreover it cannot be ruled out that the increased tumour volume might reflect an accelerated growth rate of the surviving cells (HILL & BASERGA 1975)

In fractionated radiation several factors determine if all tumour cells will be destroyed or not

Repair of sublethal injury is usually considered to take place within hours and at least before the next fraction in daily irradiation schedules The size of the absorbed dose necessary to overcome the level of only sublethal injury was demonstrated by ELKIND & SUTTON (1960) as the initial shoulder of the cell survival curve in in vitro setups The cell survival curve which is usually obtained by plot ting the proportion of cells in a population that sur vive to divide against the absorbed dose was often assumed to have a zero initial slope (Fig 5 curve A) as originally described by ELKIND & SUTTON However many data indicate that the initial slope may be non zero (Fig 5 curve B WAMBERSIE & DUTREIX 1974) The problem was extensively discussed by ELKIND (1976) Considerable evidence indicates a wide variation among different tumours with re spect to the ability to repair sublethal injury Some tumours e g malignant lymphomas are usually on clinical grounds considered to have relatively nar row shoulders and then even small daily absorbed doses are useful (LANDBERG et coll 1973 FRIEDMAN 1975) Some tumours probably have broad shoulders An example of this is malignant melanoma where conventional fractionation with a daily absorbed dose of 2 0 Gy may be ineffective (BARRANCO et coll 1971 THOMSON et coll 1975) and larger absorbed doses of the order of 4 8 Gy at each fraction are being explored (ROFSTAD et coll 1977)

Repopulation by tumour cells between successive fractions could be expected to present a clinical problem in tumours with a very high growth rate This has been shown to be the case for some un differentiated squamous cell carcinomas (FRIEDMAN) and is also observed in Burkitt s lymphoma where conventional daily fractionation has proven insufficient whereas a schedule with several frac tions each day superfractionation has proven to be effective (NORIN et coll 1971) In most other malignant lymphomas however repopulation be tween fractions seems to be of little importance the total treatment period may well be extended over a long period of time for one and the same total absorbed dose (KAPLAN 1966 LANDBERG et coll)

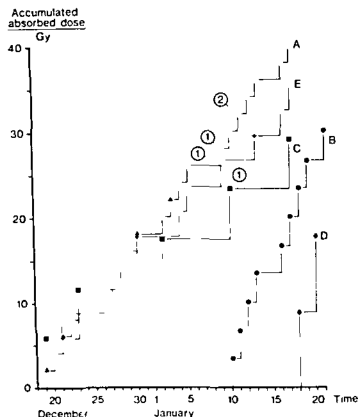


Fig 3

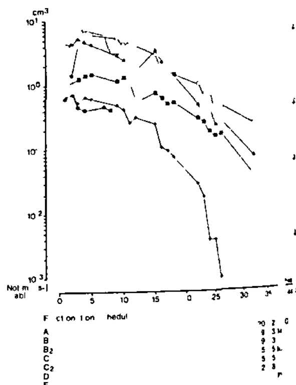


Fig 4

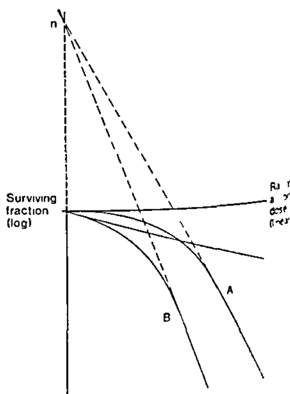


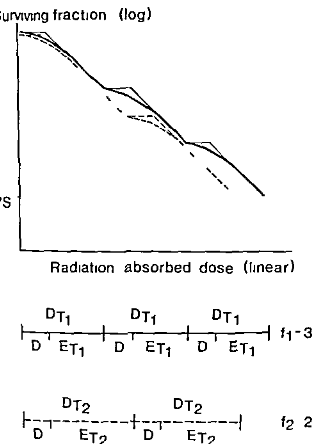
Fig 5

Fig 3 In re ise in accumulated absorbed dose with different frac tionation chedule and different kinds of skin reactions when first ob erved and mo t marked (only for sites irradiated with electrons) erythema (= 1) and dry desquamation (= 2) For expla nation of symbols see Fig

Fig 4 Size of the different tumours when measured at the begin *ning of during and after radiation treatment The uncertainties* in the volume mea urements are indicated in the right hand margin of the figure For explanation of symbols see Fig

Fig 5 Cell survival curves A with initial slope zero and B with initial lope n n zero (see review by E KISD 1976)

Reassortment of cells into different phases of the cell cycle making surviving cells enter a period of incre ised proliferative activity has been described for human tumours Thus TERZ et coll (1977) showed th it during radiation therapy of s... mours a significant increase in the thymid... porting cells occurs which was considered a... cre ised proliferating activity of the tum...



ig 6 Schematic presentation of two different radiation frac onation schedules giving the same final cell population survival fractions S) D_T absorbed dose at each fraction for schedule D absorbed dose necessary to overcome the shoulder of the ell survival curve E_T effective absorbed dose at each frac ion ($=D_T-D$) for schedule 1 f number of fractions for chedule I

opulation However this phenomenon seems to be of only limited importance during a fractionated reatment over a long time (FOWLER 1972)

/ *Reoxygenation* of hypoxic cells may be of impor ance causing a tumour to respond better (other actors unchanged) if the total treatment period is prolonged

The si e of the tumour is of importance because a larger tumour in comparison with a smaller one ontains more tumour cells relatively more resting ells (G phase STRAUS & MORAN 1977) and also may be assumed to contain larger hypoxic regions THOMLINSON & GRAY 1955) That a clear correla ion exists in the clinical situation between tumour ize and dose necessary for local control has been hown by FLETCHER (1973) for different squamous ell carcinomas and adenocarcinomas

The absolute preirradiation tumour size does not predict the dose needed but how this size has been reached seems to be of importance Thus carcinomas of the oropharynx and maxillary antrum being shrunk before irradiation with an intra arterial infusion of methotrexate had not a better local control rate than far more advanced lesions with the same total absorbed dose (NERVI et coll 1978)

The response to irradiation may vary considerably between the metastases from the same primary tumour and probably even within the same metastasis Such a variation in sensitivity by a factor of 2 or even more has been demonstrated by FRIEDMAN and TERZ et coll On the other hand STRAUS & MORAN demonstrated but little interlesional heterogeneity in cell cycle distribution in patients with solid tumours (long standing tumours) FOWLER remarked in his analysis of the results of SHUKOVSKY (1970) that they supported the simple cell survival curve theory the variation in sensitivity being small

An attempt was made to use the present observations to estimate the size of the shoulder of the cell survival curve some reasonable assumptions having been made It must be assumed that the different tumours did not differ with respect to biologic characteristics and that as a first approximation the effects of repopulation reassortment and reoxygenation were the same in the different metastases These assumptions are implicit in the calculation of the tumour volume doubling time T_d Furthermore if the tumour volume is supposed to be proportional to the number of tumour cells then the time needed for doubling the number of tumour cells is T_d as well Apparently none of these assumptions can be substantiated by data which demonstrates a common difficulty in clinical radiation biology Most biologic work on the effect of radiation has been performed with in vitro cell systems and animal tumours This can never tell what happens in vivo in man and has even led to some misconceptions in the past (RUBIN & SCARANTINO 1978) Thus data from human beings are needed although due consideration must be given the several assumptions made in the evaluation of the results as well as the clinical situation As FRIEDMAN rightly stated

Most published isoeffect curves of human cancer are based on impressment of data somewhat against their consent into a formula by the expedients of accepting data uncritically overlooking the enormity of the standard error and plotting data directly into logarithmic paper as to appear scientific

The present observations indicate that the target absorbed doses given seem to have been at least

sufficient to eradicate the metastases but insufficient to cure the much larger primary lesion The overall treatment times for the primary tumour and the four tumours A C_1 C and E are the same hence these metastases were chosen in the estimation of the shoulder in order to minimize the number of varying parameters During the period the relative changes in volume of these four tumours are approximately the same (Fig 4) The curves do not only illustrate tumour cell killing but also that other factors may be of importance e g variation in tumour cell loss and stromal tissue reactions For the present purpose it is assumed that such factors are of the same relative importance for the different sites allowing for a comparison with respect to tumour cell killing if certain precautions are taken Since the difference in initial size of the tumours A and C_1 is insignificant and it is reasonable to assume that the fractionation schedules used in the treatment of these two tumours finally result in the same surviving fractions consequently also equal absolute numbers of tumour cells survive The same situation applies for the two tumours C and E The fractionation schedules for the tumours C_1 and C are identical and according to the assumptions made this means equal surviving fractions for these tumours Thus the surviving fractions may be assumed to be equal for all four metastases the variation in initial sizes being within a factor of 5

The assumption that equal surviving fractions are reached using the three fractionation schedules A C and E makes it possible to estimate the quasithreshold dose D i e the width of the shoulder of the single cell survival curve The quasithreshold dose D is the dose at which the extrapolated high dose straight part of the dose-response curve cuts the dose axis when a presentation as in Fig 5 is used Thus the dose D is determined solely by the behaviour for large doses of the cell survival curve

Two fractionation schedules resulting in the same surviving fraction S are compared in Fig 6 The doses absorbed at each fraction are denoted D_{T1} and D_T and the number of fractions f_1 and f_2 respectively The doses D_{T1} and D_T are presumed to be large enough to result in surviving fractions characterized by the straight part of the dose-response curve This is the case in all the fractionation schedules analysed The dose absorbed at each fraction D_T can be considered is divided into two parts where D is the dose needed to overcome the shoulder and E_T is the effective dose

Table
Total effective absorbed dose and volume (the ...

Tumour	Effective absorbed dose (Gy)	Initial volume of the tumour (cm)
A	25 20	3 68
B_1	23 40	8 66
B_2	23 40	0 64
C_1	25 30	4 63
C	25 30	1 03
D	16 32	1 64
E	26 50	0 65
Primary tumour	25 20	65 0 (re urred)

$$D_T = D + E_T$$

When the same surviving fraction S is reached ... effective doses delivered by the two different treatment schedules are equal that is

$$f_1 \times E_{T1} = f \times E_T$$

which means

$$(D_{T1} - D) \times f_1 = (D_T - D) \times f$$

The width D of the shoulder is then given by

$$D = \frac{D_{T1} \times f_1 - D_T \times f}{f_1 - f}$$

No conclusions about the specific shape of ... shoulder can be made in such an analysis In fa... if the condition on the size of the doses is ... then the initial shape of the cell survival cur... arbitrary

Comparison between the schedules for tumours ... and C_1 results in the value $D_{AC} = 0\ 73$ Gy for ... shoulder dose tumours C and E give th... $D_{CE} = 0\ 93$ Gy and the schedules A and E give D... 0 58 Gy Thus the mean value $D_{EFF} = 0\ 74$ Gy ... estimation of the shoulder of the cell survival c... based on equal surviving fractions in tumours ... C_1 C and E Using the calculated value of ... shoulder total effective target doses can be ... mined These effective doses are given in the Table

The primary tumour had a volume 20 to 100 ... larger than the four tumours and was treated ... cally to tumour A Even if the same surviv...

on of tumour cells was reached in the primary as the smaller tumours the absolute number of surviving cells was higher leading to the recurrence the primary tumour It is generally considered necessary to administer a higher dose to eradicate a larger tumour For instance calculations according to COHEN s model (1971) give for a squamous cell carcinoma when a total dose of 60 Gy is given in 30 fractions over 41 days a 93 per cent chance of lethal effect on a tumour with a diameter of 1 cm while this chance is reduced to 75 per cent for a tumour with a diameter of 7 cm (350 times larger volume) A total dose of 67 Gy should be needed in order to reach the level of 93 per cent for this larger tumour the number of fractions and the over all time being the same FOWLER stated that a subclinical metastatic deposit 1 mm in diameter containing 10^6 cells can be eradicated by a dose which is two thirds as high as that required to eradicate a primary tumour of 10^9 cells

FRIEDMAN stated that accelerated recovery from sublethal injury occurs in some squamous cell carcinomas when the destructive effect on the tumour is mild either because of a low daily dose (1 0 Gy) or because the tumour is very resistant

The shoulder width arrived at seems reasonable from general clinical impressions

The results of different well defined in vitro experiments may only be clinically useful if they are properly applied to the complex in vivo situation What properly is can only be evaluated from observations in clinical work where on the other hand an investigation can be performed only if the end result is ethically acceptable Therefore deductions from a clinical investigation should be viewed with the greatest caution

SUMMARY

Subcutaneous metastases from an oesophageal carcinoma were irradiated using different schedules The results have to be evaluated with greatest caution but indicate that with the same CRE value few fractions caused less skin reactions than several and the size of the shoulder of the cell survival curve was of the order of 0 7 Gy

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FROM THE DEPARTMENT OF MEDICAL PHYSICS KING FAISAL SPECIALIST HOSPITAL AND RESEARCH CENTRE RIYADH SAUDI ARABIA

DEPTH DOSE DATA FOR 4 MeV LINEAR ACCELERATORS WITH LEAD OR URANIUM FIELD FLATTENERS

R NAIR and D E WREDE

Precise dosimetry data is a prerequisite for an effective treatment planning system in radiation therapy Per cent depth dose data obtained from two MeV linear accelerators—one equipped with lead and another with depleted uranium as field flatteners—are compared with published values This comparison clearly establishes that variations exist between depth doses which become very significant at depths greater than 10 cm of water

Material and Method

Measurements were made on two Varian Clinac 4 linear accelerators one an isocentrically mounted unit equipped with a lead field flattener and the other a stationary unit equipped with depleted uranium as the field flattener A water phantom of size 66 cm×60 cm×66 cm and a Baldwin Farmer chamber of 0 6 cm³ volume connected to a Keithly 616 digital electrometer in conjunction with a model 6169 ion chamber interface were used for measurements The calibration of the system was in accordance with the National Bureau of Standards i e the Baldwin Farmer chamber was calibrated against ⁶⁰Co and a Cλ of 0 94 was used Each value reported is the average of 4 measurements

Results

The measured central axis per cent depth dose values for 4 MV roentgen rays equipped with a lead field flattener for a few selected depths are compared with values in the literature as indicated for a 10 cm×10 cm field size at 80 cm SSD in Table 1 The per cent difference when the values are compared with those in Brit J Radiol Suppl No 11 appear in Table 3 The per cent depth dose values flattened by a uranium field flattener are given in Table 2 The per cent difference in the values when compared with those of Brit J Radiol Suppl No 11 is listed in Table 4

Conclusion

The 4 MV roentgen rays flattened by a uranium field flattener have slightly higher penetrability and in addition give improved field features and dose homogeneity within the beam (NAIR & KARTHA 1978 NAIR et coll 1978) when compared to 4 MV roentgen rays using a lead field flattener Values given in Brit J Radiol Suppl No 11 cannot be taken for granted for 4 MV roentgen rays with different field flatteners since these values show that the error can be as much as 6 per cent compared with the present results and as much as 12 per cent compared with other published data when uranium field flatteners are used assuming depths greater than 10 cm Thus direct measurement of individual beam parameters even in accelerators with supposedly similar characteristics must be emphasized

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Table 3

Percentage differences relative to Brit J Radi l depth dose values for 4 MV roentgen axis (lead field flattener) 10 cm×10 cm at 80 cm SSD

Depth (cm)	Present values	Manufacturer s data	McCullough	Peterson & Golden	Castro & Kopenhaver
2	−1 0	−1 9	−0 6	−0 4	+0 8
5	0 0	−0 5	+0 6	0 0	−1 1
10	+1 3	+1 7	+1 8	+0 5	−1 0
15	+0	+0 5	+2 1	+0 2	−3 9
20	+0 3	+0 6	+1 9	−0 3	−6 3
25	−0 4	+3 1	–	−0 9	−3 5
30	−0 6	+6 1	–	−1 8	–

Table 4

Percentage differences elative t Brit J Radi l depth dose al es 4 MV roentgen r is (uranium field flattener)

Depth (cm)	10 cm×10 cm at 80 cm SSD				20 cm× 0 cm at 80 cm SSD			
	Present values	McCullough	Review Committee		Present values	McCullough	Review Committee	
			Filter No 4	Filter No 6			Filter No 4	Filter No 6
2	+0 3	−0 7	–	–	−0 1	−0 9	–	–
5	+1 2	+0 4	−0 1	+0 7	+0 8	+0 8	+0 5	−0 8
10	+2 8	+2 0	+1 5	+ 5	+3 3	+3 8	+3 0	+ 7
15	+3	+3 4	+ 7	+3 7	+5 3	+6 3	+5 9	+5 5
0	+4 4	+5 1	+5 1	+4 1	+6	+8 5	+7 3	−6 5
25	+3 5	–	+6 6	+5 3	+4 2	–	−8 7	+7 2
30	+3 0	–	+8 5	+8 5	+4 6	–	+9 1	+11 7

SUMMARY

Per cent depth dose data for a few selected depths for 4 MV roentgen rays from Varian Clinac 4 linear accelerators equipped with lead or uranium field flatteners are presented and compared with published data A difference by as much as 12 per cent was found

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OM THE DEPARTMENTS OF RADIATION PHYSICS AND ORTHOPAEDIC SURGERY I UNIVERSITY OF GOTHEN BURG SAHLGRENSKA SJUKHUSET S-41345 GOTHENBURG SWEDEN

DUAL PHOTON ABSORPTIOMETRY IN LUMBAR VERTEBRAE

Evaluation of the baseline error

B O ROOS T H HANSSON and H SKOLDBORN

Dual photon absorptiometry is a method of de rmining the bone mineral content in lumbar ver brae in vivo (ROOS & SKÖLDBORN 1974 ROOS 975) The method is based on the following princi es

Two nuclides ^{241}Am with a gamma energy of 6 keV and ^{137}Cs with a gamma energy of 662 V are placed together under the examination ouch The patient lies on his back on the couch hich travels in the transverse direction The colli ated gamma radiation beam which c ains both hoton energies is absorbed and partly scattered by e patient s skeleton and soft tissue The ransmit d fractions are detected with a NaI(Tl) detector laced in a frame over the patient Stationary trans ission measurements are performed at a series of oints along a line transversally over the patient at e level of the third lumbar vertebra The bone ineral mass m_B (in g/cm²) is calculated for each easurement point and plotted as a function of the osition giving a bone profile curve Integration ver a subjective baseline between two end points ves the bone mineral content in g/cm

By means of dual photon absorptiometry a two hase system e g bone mineral and lean soft tissue an be solved in a stationary measurement The ttenuation for adipose tissue differs from that of an soft tissue causing a reduction of the registered one mineral mass m_B at each individual me isure ent point (ROOS 1974) This systemati measure ent error has also been observed and discussed in connection with the more common single photon absorptiometry technique (WOOTEN et coll 1973 JUDY & VOGEL 1974) With both single and dual photon absorptiometry the scanning technique with integration of the bone profile curve is normally used to correct for a layer of fat which has a constant mass along the measurement path The only influ ence of the fat in this case is a vertical shift of the bone profile curve

Considerably more serious is the error caused by an inhomogeneous distribution of adipose tissue along the measurement path A measurement path over L3 will generally pass through the caudal parts of the kidneys and thus the adipose capsules (Fig 1) All measurement points on either side of the verte bra will be influenced by the adipose capsule in the bone profile curve m_B in these areas will have a negative value It is thus reasonable to assume that the end points of the baseline (E_1 and E in Fig 1) will also have negative values (assuming that the fat in the adipose capsules of the kidneys is the only inhomogeneously distributed fat) After integration the bone mineral content will be overestimated by an amount which is approximately equal to the area A_3

In order to test this hypothesis a series of meas urements on the bone mineral content in the third lumbar vertebra was carried out in a number of cadavers using exactly the same technique as is

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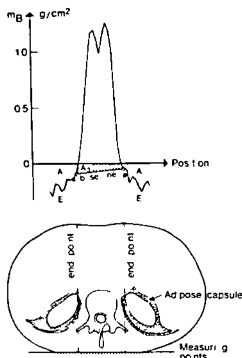


Fig 1 Influence of inhomogeneous adipose tissue distribution along the measurement path Negative values of bone mineral mass m_B due to influence of fat form the areas A and A_1 The integrated area between the end points E and E_1 represents the bone mineral content which will be overestimated by the area A

used in vivo A segment of the lumbar spine comprising vertebrae L2–L4 was then removed and measured under controlled conditions in a water bath The technique was the same as was previously used in determining the mineral content in vertebrae

Table

The age sex and bone mineral content in vertebra L3 in situ (BMC_i) and in vitro (BMC_v) and the difference between BMC_i and BMC_v in 14 cadavers

No	Age	Sex	BMC_i	BMC_v	$BMC_i - BMC_v$
1	54	M	4.24	4.09	0.15
2	55	M	5.69	5.16	0.53
3	56	M	3.37	3.82	−0.45
4	59	M	3.78	3.47	0.31
5	59	M	4.87	4.54	0.33
6	60	M	3.71	3.81	−0.10
7	64	F	[illegible]	3.38	0.84
8	64	M	[illegible]	3.97	0.30
9	69	M	5.09	4.50	0.59
10	70	F	3.84	3.21	0.63
11	72	M	[illegible]	8.40	−0.12
12	74	F	[illegible]	[illegible]	0.46
13	74	M	5.79	5.67	0.12
14	75	M	4.50	3.61	0.89

in relation to compressive strength (Ha coll 1979) The present report presents th of measurements on lumbar vertebrae cadavers and analyses the difference be vivo and in vitro measurements in order to l evaluate conclusions from biomechanical tests of compressive strength (HANSSON et

Material and Methods

The material comprised 14 cadaver years) The sex age and results of the ments appear in the Table The only form tion was exclusion of individuals who had malignancies or other diseases which mi the skeleton The most usual cause of d myocardial infarction

For the in situ measurements the cad placed in the supine position on the ex couch and vertebra L3 located by fluoros cordings were then made at 35 points alon urement path transversally over L3 The ment time was 0 7 min for each measurem The distance between the measurement po mm The recording for each measuremen designated m_B the bone mineral mass in was plotted as a function of position givi profile curve (Fig 1) The bone minera (BMC) was calculated from the bone profil follows The end points were selected su as the points lying closest to the profile c the vertebra which were projected free fr Normally the full width at half maximum of the curve may be considered to repr breadth of the vertebra and the end points 12 mm outside FWHM The area below between the end points is expressed by th ing equation

$$BMC=\left[\sum_{k}^{l} m_B^{(k)} - \frac{n-2}{2}(m_B^{\,1}+m_B^{\,n})\right]\times 0$$

where the end points are numbered (1) an $m_B^{(k)}$ is the bone mineral mass at a point be end points The bone mineral content me situ is designated BMC_i

Vertebrae L2 L4 were removed in one p autopsy and in vitro measurements then p over L3 using the same method as was previous in vitro experiments on the co strength of vertebrae in relation to the bon content (HANSSON et coll) i e with the

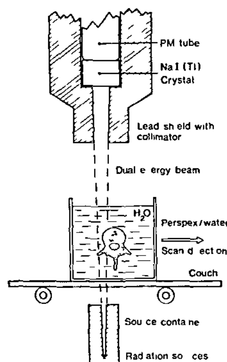


Fig 2 Experimental set up for in vitro measurement of lumbar vertebrae The preparation (L2–L4) is fixed in a water bath on the travelling examination couch and intermittent scanning is performed in the same way as in in vivo and in situ measurement

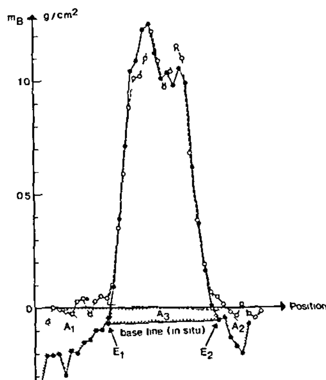


Fig 3 Bone profile curves for measurement in situ (solid line) and in vitro (dashed line) for subject No in the Table The two profile curves have been adjusted in the vertical direction in relation to the full width at half maximum of the curves The adipose effect designated A_3 in Fig 1 was in this case 0 53 g/cm

xed in a water bath as shown in Fig 2 The measurements profile drawings end point determinations and calculations of the mineral contents were made in the same way as in the in situ measurements Measurement in vitro was considered to represent the bone mineral content of the vertebra without interference from adipose tissue This value was designated BMC

The difference between BMC_i and BMC was considered to represent the systematic error caused by erroneous positioning of the baseline This difference was calculated for each vertebra and is given in the Table

Examples of bone profile curves in situ and in vitro appear in Fig 3

Results

In the Table the BMC is given in g/cm The subjects are listed in order of age The Table shows BMC_i BMC and the difference between BMC and BMC_2 It appears that the BMC difference varies from −0 45 (preparation No 3) to 0 89 (preparation No 14) The values of the BMC difference are fairly evenly distributed between these extreme values It can thus not be assumed that the BMC difference is normally distributed and a non parametric method of statistical analysis and evaluation of significance must be chosen The sign test was chosen The BMC difference in the Table has a median value of 0 32 According to the sign test the median value is significantly greater than zero with the confidence interval (0 12 0 59) at the confidence level 94 4 per cent

The median value for BMC_i is 4 39 (mean values for preparations Nos 8 and 14)

The median value for BMC was 3 90 (mean value of BMC for preparations Nos 3 and 8)

Discussion

The results thus confirm the hypothesis that in situ measurement (and thus also in vivo measurement) gives a significantly higher figure than is obtained on measurement of the same vertebra in vitro (confidence level 94 4%) This seems to be due to the fact that the end points of the baseline are

systematically positioned too low during in vivo measurement due to interference by adipose tissue on either side of the vertebra

A high correlation (r=0 86) between the bone mineral content of lumbar vertebrae and their ultimate compressive strength has previously been found in vitro (HANSSON et coll) Comparison between biomechanical properties of vertebrae in vivo and in vitro implies important potential sources of error The present results suggest that the bone mineral values in in vivo measurements systematically overestimate the mechanical strength of the vertebrae the error being of the order to 250 N (HANSSON et coll)

The differences between in vivo and in vitro measurements may have been influenced by the composition of the patient material Most of the vertebrae examined belonged to men (11/14) The amount of fat around the kidneys in men is probably greater than in individuals with osteoporosis since short slender elderly women dominate among the latter (SAVILLE & NILSSON 1966) The difference between the bone mineral content in vivo and the in vitro value in these individuals is thus probably smaller than was found in the present series (0 32 g/cm)

Based on the attenuation coefficients for adipose tissue water and hydroxyapatite (ROOS 1974) the median difference of 0 32 g/cm may be calculated to correspond to a fat mass of just over 1 4 g/cm at the end points of the baseline

SUMMARY

In connection with determination of the bone mineral content in the third lumbar vertebra by dual photon absorptiometry the fat in the adipose capsules of the kidneys is assumed to cause erroneous position of the baseline leading to overestimation of the bone mineral content The bone mineral content in L3 was measured in situ (BMC_i) and vitro (BMC) in 14 cadavers The difference between BMC_i and BMC was significantly greater than zero the median value being 0 32 g/cm at the confidence level of 94 4 per cent It is concluded that at correlation between bone mineral content and compressive strength in vitro the in vivo strength is overestimated by about 250 N

ACKNOWLEDGEMENT

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OM THE POLYTECHNIC OF CENTRAL LONDON LONDON W1M 8JS ENGLAND AND THE DEPARTMENT OF RADIATION PHYSICS KAROLINSKA INSTITUTET S 10401 STOCKHOLM SWEDEN

MICRODOSIMETRY

I Use of secondary electron emission

B J FORSBERG and T E BURLIN

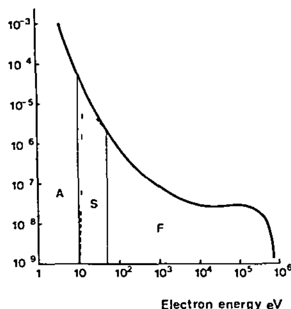
Microdosimetry is concerned with the stochastic ure of the deposition of energy by ionising radia n Since the original work of ROSSI (1959) the ject advanced rapidly at both the theoretic and erimental level The ultimate aim has been to ermine the statistical nature of the energy deposi n in biologic material particularly human tissue e experimental approaches have been dominated measurements of ionisation produced in gases ich of the theory and its associated input data rives from information based on the gaseous state uman tissue which consists largely of water ould best be modelled using the liquid state but esent techniques and knowledge do not make this ssible The solid state being a condensed phase of milar density is in principle at least as good a odel of the liquid state as gases and possibly bet r It has been suggested (BURLIN 1974) that sec ndary electron emission is a phenomenon which ould lend itself to microdosimetry This report esents an exploration of this suggestion

Secondary electron emission as an alternative technique

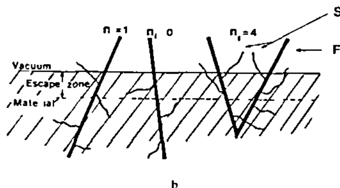
When a medium is irradiated by roentgen rays rays or electrons a degradation spectrum of elec rons is established within the medium This spec rum covers an energy range from zero up to some maximum energy Selecting the bottom of the con luction band as a suitable reference level in a conducting material the general shape of the energy spectrum is represented in Fig 1 (MCCONNELL et coll 1966) Now consider what happens when this electron spectrum passes through the interface between the material and a vacuum The electron spectrum may be conveniently divided into three energy regions (Fig 1a) First there is an energy band A where the electrons will not have sufficient energy to cross the potential barrier at the surface Secondly an energy band S exists where some of the electrons will penetrate the potential barrier and escape from the surface with an energy of 0–50 eV These only will be referred to as secondary electrons in the following The cut-off at 50 eV is of course arbitrary but has been widely accepted in the literature of secondary electron emission Thirdly there is an energy band F of fast electrons which will penetrate the potential barrier and emerge from the surface virtually unmodified In secondary electron literature this part of the degradation spectrum is often referred to as rediffused primary electrons and elastically reflected primary electron The potential barrier modifies the energy spectrum of the secondary electrons S so that outside the medium their energy spectrum is as shown by the broken line in Fig 1 The secondary electron spectrum has a marked peak at 1 to 3 eV outside the medium

The secondary electrons have a very limited range in a solid due to their interaction with the electrons

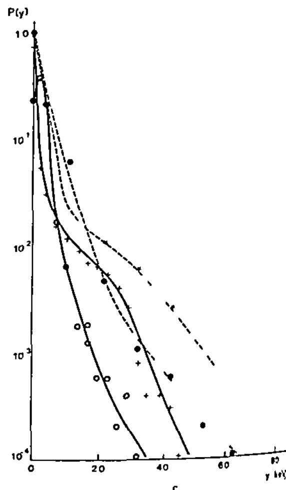
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a



b



c

Fig 1 a) Degradation electron energy spectrum ... radiated medium Broken line represents the electron e... spectrum external to the medium b) Generation of sec... electrons S by fast electrons F n is number of sec... electrons liberated by the 3 fast electrons shown c) Prob... distribution of lineal energy from the emission of secondary ... trons reflected from thick targets of carbon and lithium f... bombarded with 6 keV electrons k=1 carbon (●) k=1 ... (○) k=16 carbon (★) k=16 LiF (+)

and phonons so that those secondary electrons emitted originate in a very thin surface layer from which secondary electrons are able to escape This layer is known as the escape zone Beyond this depth no secondary electrons as defined will escape Its thickness is a characteristic of the particular material but is independent of the energy of the primary radiation The depth of the escape zone is up to 10 nm for metals (DJATLOWITSKAJA 1948) and about 50 nm or more for insulators (NAKHODKIN & ROMANOWSKY 1958 BRONSHTEIN & SEGAL 1960 BRONSHTEIN & FRAIMAN 1961) Secondary electrons emerging from a surface therefore reflect the energy deposition within volumes of linear dimensions of approximately 10 nm Thus it is possible to analyse energy deposition in volumes very much smaller than those which can be simulated by con... ventional proportional counter techniques w... are limited to volumes of linear dimensions ... proximately 0 5 μm and above

Energy deposition and secondary electron e... sion are however a stochastic process illustr... diagrammatically in Fig 1b by three fast elect... Each fast electron passing through the escape z... (in a few cases more than once) constitutes an e... in which different amounts of energy are depo... into the escape zone The energy deposited ... duces secondary electrons in the escape zone s... of which are emitted from the surface This k... fluence (there is no stochastic analogue of fl... defined so the term local has been introduced ... secondary electrons which may be meas...

ts the fluctuation of energy depositions within escape zone

Clear analogies exist between the measurement of isation in a gas and the measurement of secon ry electrons emitted from the surface of a solid

1) The sensitivity volumes (the volume from ich the ionisation is collected in the case of a seous detector and the escape zone in the case of a lid surface emitting secondary electrons) may be versed by a complete charged particle spectrum me originating at large distances from the sen ivity volume

(2) The number of electrons liberated by a primary rticle of fixed energy and direction in the sensitivi volume is subject to statistical fluctuations If the imary particle is an electron the number of elec ons liberated in the sensitive volume in a normal oportional counter or from the escape zone of a tal or insulator is small often zero The number electrons liberated in a single event (associated ith one primary particle) in a gas or solid is there re not related to the energy deposition from that ent in the sensitive volume by a simple constant of oportionality

(3) Nevertheless it is possible to identify a con ant of proportionality which when averaged over very large number of events relates the electric arge released to the energy deposition in the ensitive volume In the case of the gas the constant proportionality *is the average energy expended in* e gas per ion formed W This average embraces echanisms of energy loss other than ionisation ich as excitation by the primary particle In the se of the solid the constant of proportionality is e average energy expended in the escape zone per condary electron emitted W_{SE} This average em races mechanisms of energy loss by the primary article within the solid other than secondary elec on emission including various forms of excitation

(4) The stochastic quantity lineal energy y is the uotient of the energy imparted to the matter in a olume during an energy deposition event by the ean chord length in the volume In the case of the as the ion pairs produced are measured and multi lied by a constant W to determine the lineal ener) spectrum In the case of the solid the secondary lectrons released from the surface may be meas red and multiplied by a constant W to determine e lineal energy spectrum

Some differences exist also between the two techniques First the escape zone of a solid is effec tively an infinite thin slab rather than a finite domain and may thus simulate a membrane rather better than a gas volume Secondly unless a plate of iden tical material is placed close to the surface emitting secondary electrons electronic equilibrium is un likely to exist as is often the case with proportional counters This is not an objection in principle to the secondary electron emission technique but the spectrum of fast charged particles crossing the inter face would have to be carefully considered in any experiment

Although not undertaken in a microdosimetry context some experiments on the stochastics of secondary electron emission have been performed and the techniques would appear to be no more formidable than those currently used in micro-dosimetry For example MCDONALD et coll (1973) operated with a vacuum of 10^{-8} torr which is a relatively easy vacuum to obtain with modern techniques The secondary electrons emitted from a surface were accelerated through 45 kV to a lithium drifted silicon detector pulse amplifier with a resol ution of 3 1 keV The number of secondary elec trons per event was recorded by a multi channel analyser and this is the basic data of microdosimetry from which y spectra and mean values can be ob tained They also undertook Monte Carlo calcula tions of energy deposited within an escape zone and the associated secondary electron emission which *agreed well with their measurements*

Stochastic aspects of secondary electron emission

When a material is irradiated secondary electrons will be emitted from the surface The literature de scribing various secondary electron emission phe nomena is extensive but work concerning the stochastic aspects of the emitted secondaries is sparse Some of the work reported is presented in Table 1 They all describe measurements or calcula tions of the probability distributions P(n) of the number of secondary electrons emerging per inci dent fast electron from different surfaces

There is some concentration in the choice of materials and energies of the primary particles ex amined This is because the materials investiga ed (alkali halides) are all common as sensitive layers in dynodes in photomultipliers and in the energy range 6 to 8 keV maximum yield of the secondary elec trons occurs for the thickness commonly examined

Table 1

Investigations concerning the stochastic of secondary electron emission

Reference	Material	Radiation	Calculation	Measurement
DELANEY & WALTON (1966)	Al	5 3 MeV α particles	No	Yes
DIETZ et coll (1966)	KCl KCL + AlO	8–9 keV electrons 25 keV ions	Yes	Yes
PRESCOTT (1966)	General	Electrons	Yes	No
LLACER & GARWIN (1969 b)	CsI KCl LiF	^{90}Sr–^{90}Y β rays 9 keV electrons	Yes	Yes
WILCOCK & MILLER (1969)	CsI KCl	$\leq$10 ke V electrons	No	Yes
GREUPNER (1972)	Al Cu etc	Positive ions	No	Yes
MCDONALD et coll (1973)	CsI KCl NaCl LiF	5 9 keV roentgen rays	Yes	Yes
CAFOLLA et coll (1975)	CsI KCl LiF	$\leq$5 keV electrons	Yes	Yes

One of the materials (LiF) can be regarded as near tissue equivalent as far as its absorption characteristics for directly and indirectly ionizing particles are concerned LLACER & GARWIN (1969 b) have made measurements of the secondary electron emission from different materials irradiated with β rays (^{90}Sr–^{90}Y) and calculations with 1 MeV and 7 to 9 keV electrons as primary particles They employed a dielectric theory to account for the energy losses and the electron–phonon interaction to determine the probability that a second electron will reach the surface and escape They suggest the following formula for this escape probability

$$P(\varepsilon_0\ x) = P_0(\varepsilon_0\ 0)\exp(-\ /L)$$

where $P\ (\varepsilon_0\ x)$ is the probability that an electron generated with an energy ε_0 above the conduction band at depth x will reach the surface and escape and L is a characteristic escape length A more accurate formula for the escape probability was obtained by weighting the sum of exponentials over the energy spectrum of the secondaries

MCDONALD et coll (1971 1973) have developed a Monte Carlo program with which they make calculations of the electron transport through different materials The probability of escape is determined in the same way as by LLACER & GARWIN The probability $P(\varepsilon_0\ 0)$ of an electron of energy ε_0 situated at the surface (x = 0) escaping was equal to 0 75 for all their calculations Therefore they had only to choose the overall energy loss corresponding to the production of one internal secondary electr… and the characteristic escape length L for e… material Their computations were in good agr… ment with measurements made using the 5 9 k… roentgen rays from ^{55}Fe

While most authors have calculated the secon… electrons emerging from the exit surface of a… CAFOLLA et coll (1975) have made calculati… the secondary electrons emerging from the e… surface Experimental results employing low e… electrons concur with these calculations

Theoretical exploration of technique

The calculations are based on a Monte C… program described in detail by MCDONALD et… (1971 1973) (see also GREEN 1963 and OSTR… HOV 1967)

The first electron tracks are divided int… ments Each segment is terminated by an el… Rutherford scattering from the nucleus wh… governed by the equation

$$\frac{d\sigma}{d\Omega}=\left(\frac{Ze^2}{4E}\right)\ \left(\sin^2\frac{\theta}{2}+\frac{\vartheta}{4}\right)^2$$

where $\frac{d\sigma}{d\Omega}$ is the differential cross section per solid angle

Z is the atomic number of the absor… material

e is the electronic charge

E is the instantaneous energy of the e… tron

θ is the angle of deflection

ψ is a correction factor to the simple Rutherford formula to take account of the screening effect of the orbital electrons when the incident electron passes the nucleus at a large distance

Three conditions of randomness are introduced each elastic scattering by the following three onte Carlo equations

$$\frac{\varphi}{2\pi}=\gamma \quad (2)$$

$$\frac{\int^{\theta}(d\sigma/d\Omega)\sin\eta\,d\eta}{\int_{\pi}(d\sigma/d\Omega)\sin\eta\,d\eta}=\beta \quad (3)$$

$$\exp(-x/\lambda)=\alpha \quad (4)$$

here α β and γ are random variables uniformly istributed in the interval (0 1) Eqs 2 and 3 give the irection of the new segment of the track with respect to the previous one Eq 2 indicates that the hoice of φ the other polar co ordinate (with θ) efining direction is arbitrary Eq 3 allows a random choice for θ which accords nonetheless with the general angular distribution of eq 1 Eq 4 allows the selection of a random distance x to the next ollision (i e the length of the next segment) λ eing the mean path length

The energy loss in a segment of electron track is etermined by the Bethe formula

$$-\frac{dE}{dx}=\frac{4\Pi e^4 NZ}{mv}\ln\left(\frac{mv}{2I}\right)\sqrt{\frac{1}{2}e} \quad (5)$$

his energy loss in the segment is attributed equally o distant and near collisions The half of the nergy attributed to distant collisions produces xcitation of the atoms concerned and is considered o be deposited in that segment of the track The half of the energy attributed to near collisions produces onisation and results in collisions occurring ran lomly once in every k segments McDONALD et oll (1971) chose the value of k by obtaining a good it to the experimental energy spread of electrons merging from an aluminium foil Their value of k= 16 is also employed in the present calculations Thus the energy attributed to near collisions may be leposited in another segment of track together with nergy from near collisions from k segments There are a very large number of distant collisions with very small energy loss per collision The number of near collisions are few in number with a large energy loss per collision though the energy loss per collision is still small compared with the energy of the fast electron Thus secondary electrons are produced only in segments of tracks where near collisions occur The number of secondary electrons produced is determined by dividing the energy lost in the near collision by the average energy required to produce a secondary electron in the material

The interaction of these secondary electrons which have very low energy is predominantly with the phonons As already noted the probability that a secondary electron generated with energy ε_0 at a depth x will reach the surface and escape is $P_0(\varepsilon_0\ 0)\exp(-x/L)$ Strictly both $P_0(\varepsilon_0\ 0)$ and L are functions of initial secondary electron energy and should be appropriately weighted over the energy spectrum of the secondaries However adapting average values for each material has proved completely satisfactory (McDONALD et coll 1973)

The probability P(n) that n secondary electrons will emerge per primary electron was calculated with k=1 and k=16 When this is multiplied by the average energy expended in the escape zone per secondary electron emitted the probability distribution of the number of events in event size P(y) is obtained Thus the measurement of the secondary electrons illustrated in Fig 1 b can provide the probability distribution of lineal energy deposited in the escape zone as is illustrated in Fig 1 c Hence the frequency mean and the dose mean of lineal energy may be obtained A continuous energy loss model for electrons is represented when k=1 The probability distribution will then only be caused by the differences in the track lengths (sum of track segments) of the electron within the escape zone and the relative variance can be shown to be proportional to ln (constant/L) Thus as shown in Fig 1 c the relative variance for lithium fluoride which has a thick escape zone is less than for carbon which has a thinner escape zone A discontinuous energy loss model for electrons is represented when k=16 and again the relative variance due to energy loss straggling is greater for carbon the material with the thinner escape zone

Following McDONALD in these calculations k= 16 was employed Calculations of the probability distributions were carried out for monoenergetic electrons with initial energy between 6 and 30 keV directed normally towards infinite thick targets and the distributions were determined at the entry side

Table 2

Input parameters to the calculations I is the average excitation ionisation potential Z is the atomic number L is a characteristic escape length ε is the energy loss corresponding to the production of one internal secondary electron P (ε₀ 0) is the probability that an electron having an energy ε above the conduction band at zero depth will escape

Material	I (eV)	Z	L (nm)	ε (eV)	$P(\varepsilon\ 0)^h$	W_{SE} (eV/electron)
Carbon	78	6	1 3[d]	11	0 22	61[f]
Lithium fluoride	87 2	8 2	8 0[c]	36	0 80	66
Aluminium	164[b]	13	1 3[d]	13	0 60	35[f]

ICRU Report 16 (1970)
BISCHEL (1968)
LLACER & GARWIN (1969 a)
BRONSHTEIN & SEGAL (1960)
FITTING et coll (1976)
KANTER (1961)
Data for primary electron energies below 10 keV
Best fit to experimental results of KANTER (1961)

Similar calculations were also made for electrons up to 60 keV directed towards thin films of different thickness and the distributions were determined at the exit side

Choice of parameters The different parameters used in the calculations are listed in Table 2 A comparison of these calculations with experimental measurements is presented in Fig 2 and is taken to indicate that the choice of input parameters is reasonable The sources of these input parameters are described in the following

The overall energy loss corresponding to the production of one internal secondary electron ε of 36 eV for LiF was taken from LLACER & GARWIN (1969 a) though it is noted that McDONALD et coll (1973) obtained a best fit to their experimental results using 48 eV Below a primary electron energy of 10 keV the value for aluminium was derived from FITTING et coll (1976) who calculated the secondary yield inside a target per primary particle and knowing the energy dissipated in the target derived ε values for different materials An interpolation from their data was made for carbon For higher primary energies ε was obtained by finding the best fit between calculation and measured yield values of REIMER & DRESCHER (1977) for aluminium at higher energies employing the values of P (ε_0 0) in Table 2 An interpolation was made for ε for carbon at higher primary energies For silicon and germanium the average energy corresponding to an electron hole pair is in the range 3–4 eV this may be compared with ε for the materials listed in Table 2

The choice of the value of ε and P (ε 0) is interdependent so that increasing the P (ε_0 0) val 10 per cent will have approximately the same ef as decreasing the ε value by 10 per cent Therefr it is always possible by means of comparison w experimental data to minimise possible errors P (ε_0 0) and ε In fact P (ε_0 0) was determined b finding the best fit with experimental data of KAN TER (1961 Fig 2) Values of 0 60 and 0 22 for Al a C respectively were obtained

An alternative approach to estimating P (ε which provides a check on these values is to not that P (ε_0 0) is closely related to the express (BURLIN 1974)

$$P_0(E) = 1 - \left\{\frac{F_{mi}}{E}\right\}^{1}$$

where P_0 (E) is the probability that an electron energy E will escape from the surface and F equal to the electron affinity of the crystal for e sulators and equal to the sum of the photo-elec work function and Fermi energy for metal Fr alkali halides the electron affinity is a few ten one eV and taking from LLACER & GARWIN (19 a value of 3 eV for the mean energy of the second electrons P (E) is derived to be 0 8 If th energy electron spectrum established within re is represented with respect to the Fermi level an average energy of 8 eV a figure of the P(E) f and Al could be derived With a photo-electr function for C of 4 8 eV and for Al of 3 eV values for P_0(E) of about 0 4 and about 0

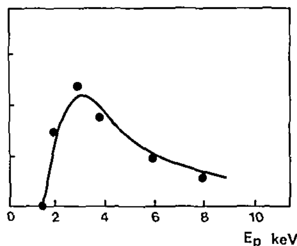


Fig 2 a

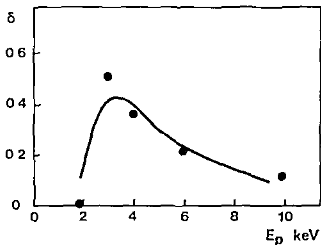


Fig 2 b

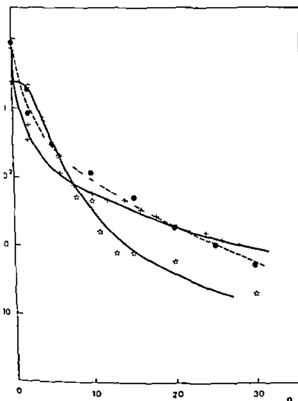


Fig 3

Fig 2 Comparison with experimental yield curves obtained from the exit side of foils by KANTER (1961) a) 17 5 μg/cm² thick Al foil b) 71 5 μg/cm² thick C foil Experimental results (—) Calculated (●)

Fig 3 Probability distributions for emission of secondary electrons from the exit side of a lithium fluoride foil bombarded with 7 keV electrons Experiment by LLACER & GARWIN (1969 b) (●) calculated k=1 (☆) calculated k=16 (+)

rived for Al and C respectively These estimates are in reasonable agreement with the values obtained by fitting the data of KANTER

A common method to determine the characteristic escape length L is to equate it to one third of the escape zone In LiF LLACER & GARWIN (1969 a) considered an electron energy of 8 eV to have a maximum escape depth of 25 *nm which corresponds* to a value of 8 nm MCDONALD et coll (1973) got a best fit with their experiments using 8 nm providing some confirmation of this value According to BRONSHTEIN & SEGAL the maximum escape depth is 10 to 20 atomic layers for metals corresponding to a characteristic escape length of 1 to 2 nm

An examination indicates that the calculations were not very sensitive to the value of L or ε Thus L was *increased by a factor* of two the dose mean of the lineal energy y_D increased with 13 per cent If ε

was increased from 38 eV to 58 eV for 6 keV electrons incident on LiF the dose mean of the lineal energy y_D decreased 10 per cent though it should be noted that increasing L by a factor of two will not result in any good fit

The average energy expended in the escape zone per secondary electron emitted W_{SE} is required to derive the energy deposition This quantity was taken for C and Al from KANTER and derived for LiF from LLACER & GARWIN (1969 a) These values are strongly dependent on the cleanness of the surfaces through which the electrons escape and should be regarded as approximate but nevertheless providing a picture of the energy deposited in the sensitive volume

Comparison with yield curves are an inadequate demonstration when the objective of the calculation is the complete P(n) distribution Therefore a calculation for 7 keV electrons directed on a thin film were compared with the experimental results of LLACER & GARWIN (1969 b) Fig 3 clearly demonstrates the effect of energy straggling Using k= 16 and the parameters listed in Table 2 the agreement between the curves present in Fig 2 c is satisfactory

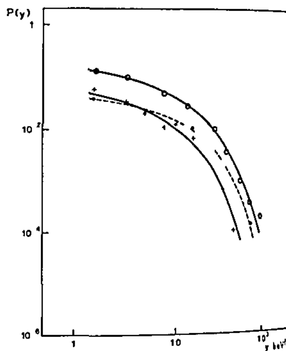


Fig 4 Probability distribution of lineal energy per unit ... lineal energy based on secondary electrons emission ... from thick targets of carbon aluminium and lithium fl... bombarded with 6 keV electrons Points show the fluctua... the calculated results For C and Al the points in the lo... energy interval are interpolated values between P(0) the ... ability of no secondary electrons emitted and the proba... exactly one secondary electron emitted For C (O) P(0) ... for Al (✱) P(0)=0 94 for LiF (+) P(0)=0 77

Results and Discussion

The objective of this report is to illustrate the potential of secondary electron emission as a technique for microdosimetry An extension of the use of proportional counters to volumes with dimensions equivalent to less than 0 2 μm in tissue presents formidable difficulties and a probable increased uncertainty in the measured quantities In contrast data are presented in Figs 4 and 5 for the lineal energy probability distributions for volumes with linear dimensions in the nm region However it should be noted that the definition of lineal energy *involves the mean chord length* $\bar{l}$ *which for a thin* slab under isotropic irradiation conditions is equal to twice the thickness (i e twice the characteristic escape length (BIRKHOFF et coll 1970) Taking the characteristic escape length as one third of the escape zone resulted in mean chord lengths in unit density material of 6 7 and 42 nm for carbon aluminium and lithium fluoride respectively Since no isotropic conditions exist the values mentioned should be considered as upper limits Fig 4 presents data relevant to secondary electrons emitted from

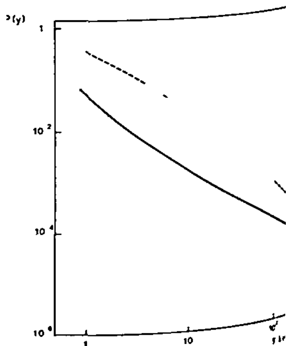


Fig 5 Probability distribution of lineal energy per unit ... lineal energy based on secondary electrons emi... side of a 95 5 nm thick carbon foil At 6 keV ... 60 keV (—) P(0)=0 ...

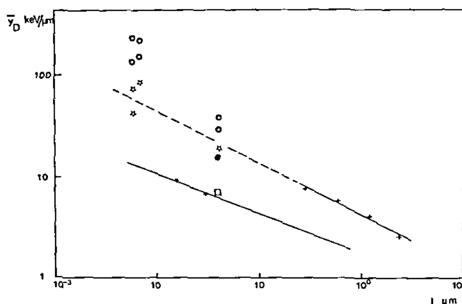


Fig 6 Dose mean of lineal energy versus mean chord length in unit density material ^{60}Co γ rays (★) BENGTSSON & LINDBORG (1974) ^{3}H β-rays (+) BRABY & ELLETT (1971) 7 keV electrons (●) and ^{90}Sr–^{90}Y β rays (□) calculated from LLACER & GARWIN (1969b) Calculations (☆ reflection) Calculations (O transmission) C corresponds to l=6 nm Al to l=7 nm LiF to l=4 nm

the entrance surface of an absorber and illustrates how the distribution can vary with the nature of the absorbing material It will be noted that the linear dimension of the sensitive volume varies with the material because of differences in thickness of the escape zone Fig 5 presents data relevant to secondary electrons emitted from the exit surface of an absorber and shows how the distribution can vary with the energy of the incident electron The incident energy of the primary electrons is indicated on Fig 5 but the energy of the electrons passing through the escape zone on the exit side of the foil will of course be less than this

These data form the basis for calculating the dose mean and the frequency mean of the lineal energy and the relative variance These values are presented in Table 3 Where data in Table 3 (and also Figs 5–7) are given for transmission the calculations have been undertaken for the foil thickness employed by KANTER and LLACER & GARWIN (1969b) They were 95 5 nm 50 nm and 64 8 nm for carbon lithium fluoride and aluminium respectively The quantities in Table 3 are defined as follows

$$y_{F\,0}=\frac{W_{SF}}{l}\,\frac{\Sigma_0\, i\, n_i}{\Sigma_0\, n_i}=\frac{W_F}{l}\left\{\frac{0\; n_0}{\Sigma_0\, n_i}+\frac{\Sigma_1\, i\, n_i}{\Sigma_0\, n_i}\right\}=\delta\;\frac{W_F}{l} \quad (6)$$

$$y_{F\,1}=\frac{W_F}{l}\,\frac{\Sigma_1\, i\, n_i}{\Sigma_1\, n_i} \quad (7)$$

$$y_D=\frac{W_{SF}}{l}\,\frac{\Sigma_0\, i^2\, n_i}{\Sigma_0\, i\, n_i} \quad (8)$$

$$V_{y\,i}=\frac{y_D}{y_{F\,0}}-1 \quad (9)$$

Where n_i events result in the emission from the surface of i secondary electrons δ is the average yield of secondary electrons per primary electron and l is the mean chord length across the escape zone

The first term in the summation for $y_{F\,0}$ represents an event which does not result in the emission of a secondary electron (this may occur either because the primary electron did not generate any secondary electrons within the escape zone or because secondary electrons which were generated failed to cross the surface potential barrier) and the second term the number of events which do

A large difference exists between the values of $y_{F\,0}$ and $y_{F\,1}$ This is because there are many events which do not lead to the emission of a secondary electron and these events heavily weight the spectrum For carbon and aluminium the probability per primary particle that a secondary electron will not be emitted is 0 95 and for lithium fluoride 0 80 for 6 keV primary electrons The mean lineal energy is always larger in the case of the transmission data for three reasons First some input data for the calculation were based on curve fitting of transmission

Table 3

Mean values and relative variance of the single event distribution

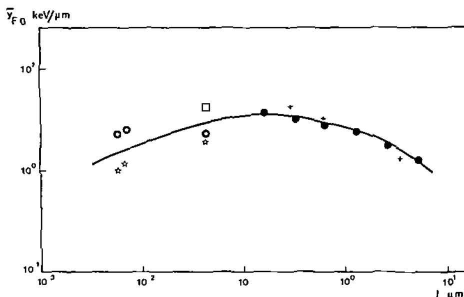
Material	Primary electron energy E_0 (keV)	Mean lineal energy (keV/μm) y_F	$y_{F,1}$	y_D	Relative variance V_1
Reflection					
Carbon	6	1.0	23.5	71	70
Lithium fluoride	6	2.1	9.2	19	8
Aluminium	6	1.2	12.9	82	67
Transmission					
Carbon	6	2.3	69.0	225	96
	60	0.2	55.0	138	690
Lithium fluoride	6	2.4	12.5	38	11
	60	0.3	12.3	28	176
Aluminium	6	2.6	57.2	214	81
	60	0.2	50.5	143	714

measurements and relative to reflection measurements (HOLZL & JACOBI 1969) the calculation tends to give low values for the yield from the incident surface. Secondly the primary electrons will have a lower energy on reaching the exit surface of the foil. Thirdly the primary electrons are normally incident on the escape zone of the entry surface but will tend to be more isotropic as they cross the escape zone of the exit surface. In contrast to carbon and aluminium the value of the mean lineal energy for lithium fluoride obtained from reflection and transmission calculation is not very different. This is because the foil thickness for lithium fluoride is 50 nm and the escape zone is 24 nm. Thus the entry and exit escape zone are adjacent or even overlapping for lithium fluoride whereas they are widely separated for carbon and aluminium. It will be noted that for these small volumes the relative variance is extremely large.

The dose mean of lineal energy y_D obtained from conventional microdosimetric measurements is compared with values obtained from secondary electron emission in Fig. 6. For low energy electrons the experimental results obtained by proportional counter techniques (BRABY & ELLETT 1971) using ^3H β rays are compared with the data in Table 3 and the secondary electron emission measurement by LLACER & GARWIN using 7 keV electrons. The measurement in lithium fluoride is very close to the value calculated by the theory of MCDONALD et coll for reflected secondary electrons. The pos[...] of the secondary electron points is of course d[...] mined by the thickness of the escape zone [...] particular material. Thus the lowest value of [...] mean chord length (i e smallest volume) is for [...] bon 6 nm and aluminium 7 nm. If the propor[...] counter results are extrapolated linearly they [...] in the graphs through the results obtained by [...] ondary electron emission giving good agree[...] with the measurement by LLACER & GARWIN [...] the theory of MCDONALD et coll for reflected [...] ondary electrons.

The experimental results obtained by BENGTS[...] & LINDBORG (1974) with the electrons produce[...] 1.25 MeV ^{60}Co γ rays are compared with a [...] rived from the secondary electron emission m[...] urement by LLACER & GARWIN using [...]Sr β rays. The measurements by BENGTSSON [...] LINDBORG extend down to 7 nm mean chord le[...] and therefore no extrapolation is necessary t[...] brace the measurement on lithium fluori[...] LLACER & GARWIN. The agreement is good (F[...] although the approximations in the estimation of [...] mean chord length have to be borne in mind [...] agreement for both high and low energy ele[...] particularly interesting when it is recalled th[...] ondary electron emission simulates a very th[...] while conventional microdosimetric techniques [...] ulate spheres or cylinders.

Further data are required on y_D values f[...]



Frequency mean of lineal energy versus mean chord length density material H β rays (+) BRABY & ELLETT (1972) rays (●) KLIAGUA & DVORAK (1978) 7 keV electrons (□) calculated from LLACER & GARWIN (1969 b) Calculations for C Al and LiF (☆ ○) as in Fig 6

gy electrons or roentgen and it is possible that a nique used by BENGTSSON & LINDBORG could pplied to secondary electron emission devices xperimental approach to this has been under n (FORSBERG 1978) and further experiment is beeing made

ne frequency mean of lineal energy y_{F0} ob d from conventional microdosimetry measure ts is compared with values obtained from sec ary electron emission in Fig 7 for low energy trons The conventional measurements by BY & ELLETT with ³Hβ rays and by KLIAGUA & RAK (1978) with electrons produced by 11 keV tgen rays show good agreement the frequency n of the lineal energy increasing slowly with reasing volume The secondary electron emis data for 6 keV electrons from Table 3 calculated McDONALD s theory and the measurement for 7 electrons by LLACER & GARWIN are also wn A continuous line has been plotted through se data At mean chord lengths below 100 nm the quency mean of the lineal energy decreases slow with decreasing volume This reflects the influence the zero events that is particles passing through volume *without* resulting in any escape of sec dary electrons It has been argued in the foregoing t secondary electron emission techniques can ld useful microdosimetric data The results are t very sensitive to the choice of ε and P (ε_0 0) r will much uncertainty arise from the choice of l and Z The greatest uncertainty in these calculations will arise from the choice of L which will influence the shape of some distributions and W_{SE} which will not influence the shape of the distributions but will affect the absolute values and hence the mean value quoted The maximum uncertainty in the abso lute values is estimated at about 50 per cent CHMELEVSKY & KELLERER (1977) have recently undertaken computation of microdosimetric func tions for sites of 5 10 and 100 nm diameter for protons of energies 0 5 5 and 20 MeV While not directly comparable with the present calculations some confirmation of the trend of the y_D values in Fig 6 can be found For protons with a comparable stopping power as 6 keV electrons they also got an increasing y_D at decreasing object sizes However the burden of this report was not to produce abso lute values but to illustrate some of the potentials of this alternative approach to microdosimetry

In conclusion the authors wish to emphasize three aspects of this technique which may offer some advantages over other physical models of biologic systems First the linear dimensions (10 nm) are comparable with what is believed to be the dimensions of biologic targets Secondly the shape is an extended layer which would simulate the situation where the sensitive structures are distrib uted in this layers for example in the vicinity of nuclear membranes (ALPER 1969) Thirdly another interesting biologic application should be the use of

secondary electron emission to examine the two target theory It has been proposed by several authors that certain biologic effects may result from energy depositions taking place in two different targets sublesions (NEARY 1965 ROSSI 1968 ALPER) NEARY suggested that these targets should be in the nanometer region and a separation distance of 0 2 μm KELLERER & ROSSI (1971) concluded that cells of higher organisms are injured by radiation through production of an ionisation in each of two targets of nanometer size with an interaction distance of micrometers ALPER proposed that cell death may occur if energy depositions are taking place both in the DNA and the nuclear membrane Most recently KELLERER & ROSSI (1978) have presented a generalised formulation of dual radiation action Aspects of the situations could be simulated by registering the secondary electrons liberated by the same primary particles from escape zones (having dimensions of about 10 nm) on the entry and exit sides of a foil having dimensions of 0 2 μm or above

SUMMARY

The potential of secondary electron emission as a basis for microdosimetric analysis is explored Secondary electron emission phenomena are compared with gaseous ionisation and a brief review of work on the stochastic nature of secondary emission is presented Input data are selected and calculations undertaken to obtain lineal energy spectra The frequency and dose mean of lineal energy obtained from these calculations in various volumes are compared with results obtained with proportional counters Some biologic applications of this approach to microdosimetry are discussed

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SIGNIFICANCE OF QUANTUM FLUCTUATIONS IN ROENTGEN IMAGING

KARL GUSTAV STRID

Optimisation of roentgen diagnostic techniques plies the development of procedures by means of ich the desired diagnostic image quality is pro ced with the further requirement that the radia n dose absorbed by the patient be kept at a nimum Image quality is limited by a number of tors which may be grouped under a few head s viz (a) geometric unsharpness (blurring due to al spot size motion blurring light spread in de ting layers etc) (b) dynamic limitations (visi ity thresholds) and (c) statistical limitations uantum noise lack of elementary receptors in tecting layers) Unsharpness is readily describ le by means of the optical transfer function of the aging system (MORGAN 1962 SCHOBER & HÖ RT 1963) whereas dynamic limitations are in rred from the overall system sensitometric func on inclusive of the contrast threshold of the human e inspecting the final image (BLACKWELL 1946) he significance of photon statistics was pointed out STURM & MORGAN (1949) the fluctuations giving e to quantum mottle (CLEARE et coll 1962 ROSS ANN 1963) Lack of receptors (e g silver halide ains) in the detecting layer further contributes to e resulting image mottle (SELIN & REICHMANN 77) which implies that secondary radiation must kept at a minimum (SELIN et coll 1975 STRID 76 REICHMANN & STRID 1979) contrary to the ew held by REISS (1963) The relation between age quality and patient dose was examined experimentally by REICHMANN & ÅSTRAND (1979) and theoretically analysed by MOTZ & DANOS (1978)

Within the scope of a program for optimising roentgen imaging techniques a series of mathematical investigations has been initiated The work will concern the statistics of the radiation relief the interaction between roentgen radiation and photographic type detectors and the interplay of unsharpness and quantum fluctuations

As a first part of the work the present report furnishes an analysis of quantum fluctuations in the radiation relief proper some implications with regard to image recording also being discussed however the behaviour of photographic type detectors will not be treated In addition the mathematical framework to be employed in forthcoming reports is provided Therefore elements of the theory of stochastic variables somewhat beyond the scope of the present report have been compiled in an Appendix

List of symbols

Other symbols than those listed below may occasionally be used in the text Additional symbols are used in the Appendix

A Area of object detail to be detected as projected on the recording plane unit 1 m

Submitted for publication 11 September 1979

C	Radiation contrast
d	Linear extent of object detail to be detected unit 1 m
D	Radiation dose unit 1 Gy
K	Constant of proportionality
N	Number of photons
p	Probability of a photon process
$P(x)$	Probability that the event x will take place
S	Signal to-noise ratio
T	Transmittance of secondary screening device
$var(x)$	Variance of the quantity x
ε	Detective quantum efficiency
ν	Repeatancy (spatial frequency) of object structure to be resolved unit 1 m ¹
$\sigma(x)$	Standard deviation of the quantity x
Σ	Selectivity of secondary screening device
Φ	Photon fluence unit 1 m
Ψ	Relative fluence of secondary radiation

Subscripts

D	Detector (recording device)
p	Primary radiation
s	Secondary radiation
t	Total radiation field
0	Incident radiation

Primed quantities e g S_1 refer to the radiation field behind the secondary screening device whereas double primed ones e g S_1 refer to the response of the detector Angle brackets e g $\langle x \rangle$ denote the mean value of the quantity described by the enclosed symbol

Basic considerations

A radioscopic situation is considered where an object is exposed to roentgen radiation of photon fluence Φ_0 (Fig 1) For the sake of simplicity—in order to arrive at a mathematical model of instructive clarity—the radiation is assumed to be monoenergetic i e composed of photons of equal energy content This restriction may be easily relieved by replacing fluence with its differential spectral distribution and integrating the relevant quantities over the entire spectrum however the clarity of the model would then be lost

On passage through the object the radiation is attenuated and behind the object a radiation field appears viz the primary radiation relief in which the photon fluence Φ_p is modulated with information regarding the distribution of matter in the object

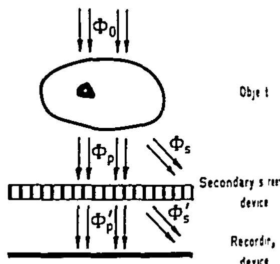


Fig 1 Flow of roentgen energy in radioscopy Incident field of photon fluence Φ is modulated by the object to an informative field of fluence Φ and a non-informative field of fluence Φ Secondary screening modifies these values to Φ and Φ respectively

ject Moreover due to scattering and fluorescence secondary radiation arises in the object appearing as another field of photon fluence Φ For the present analysis this field is assumed to be homogeneous over the imaging area considered The resulting secondary radiation relief will impair imaging as compared to the primary relief alone

In order to restore image quality some kind of discriminating device is inserted into the secondary radiation relief to produce a modified radiation field with fluence values Φ_p and Φ respectively this field being intercepted by the recording device so as to produce the image

Formation of contrast

If the radioscopic system is intended to resolve object details of a characteristic extent d (Fig …) it is relevant to consider the imaging of a periodic object of repeatancy (spatial frequency) … more specifically to analyse the contrast between two squares 1 and 2 of the same area d^2 … 1/4… one in a region of high transmittance and the other in a region of low transmittance of the object

The transmitted primary fluence is assumed Φ_{p1} and Φ_{p2} respectively Due to the … nature of the radiation these quantities refer to average flow of photons (cf ICRU 1971) The

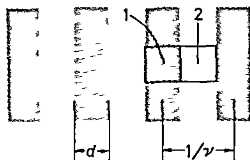


Fig. 2. Schematic representation of a periodic object of re…tancy ν. For the structure to be resolved by radioscopy the imaging system must yield significantly different responses for squares 1 and 2 of area $A=d^2=1/4\nu^2$ located respectively in regions of low and high transmittance.

the object to modulate the radiation field is then suitably described by the primary radiation contrast

$$C_1 \equiv \left|\frac{\Phi_{p1}-\Phi_p}{\Phi_p}\right| \quad (1)$$

where

$$\Phi_p \equiv \tfrac{1}{2}(\Phi_{p1}+\Phi_{p}) \quad (2)$$

The secondary radiation presents the fluence Φ_s which is proportional to the primary fluence

$$\Phi_s \equiv \psi\Phi_p \quad (3)$$

whereby the relative fluence of secondary radiation ψ is defined. The resulting radiation contrast is then changed from C_p to

$$C_t \equiv \left|\frac{\Phi_{p1}-\Phi_p}{\Phi_p+\Phi_s}\right| = \frac{C_1}{1+\Psi} \quad (4)$$

This contrast reduction, however, does not exhaustively account for the resulting image impairment since the statistical properties of roentgen radiation have been entirely neglected. Thus the conventional treatments, e.g. by OOSTERKAMP (1946), HONDIUS BOLDINGH (1960, 1964) and REISS give an incomplete description of the effects of secondary radiation.

Randomness of roentgen radiation

The emission of photons in the radiation source takes place in a completely random way, i.e. the moment and the direction of emission of a particular photon are completely unrelated to those of any photon emitted previously. Roentgen photon emission is a stochastic process of the Poisson type (cf. Appendix). Absorption, scattering and secondary emission by the radioscopic object are likewise random processes; indeed, the conversion in the object of the incident radiation field to one field of informative photons (primary radiation) and another of non-informative ones (secondary radiation) are binomially distributed stochastic processes (cf. Appendix), making the resulting fluxes Poisson distributed as well. No exact a priori predictions may be made as to the numbers of photons of primary and secondary origin propagating through a given region of space during a particular interval of time. However, the average values of these numbers can be calculated, provided the proper physical and geometric characteristics of the radiation source and the radioscopic object are known.

Signal-to-noise ratio

Consider a region in the object forming a cylinder along the direction of incident radiation of projected area A. If the probability of undisturbed transmission of a photon through the object is p_p, the fluence of primary photons leaving the object is $\Phi_p=p_p\Phi_0$, Φ_0 being the fluence of incident photons, and the mean number of photons transmitted through the region of area A is

$$\langle N_p\rangle=\Phi_p A=p_p\Phi_0 a \quad (5)$$

with the standard deviation

$$\sigma(N_p)=\Phi_p A \quad (6)$$

which follows from eq. (A 19) (Appendix). Similarly, for a laterally extended and reasonably homogeneous object, the mean number of secondary photons emitted towards the corresponding area in the recording plane is

$$\langle N_s\rangle=\Phi_s A=p_s\Phi_0 A \quad (7)$$

with the standard deviation

$$\sigma(N_s)=\sqrt{\Phi_s}A \quad (8)$$

assuming p_s to be the probability of an incident photon being turned into a secondary one travelling towards the recording plane.

Now consider the periodic object in Fig 2 The primary radiation field is modulated by the object the primary contrast being defined by eq (1) Since small variations in object transmittance are of the utmost importance in diagnostic radiology and moreover present the greatest recording difficulties the assumption of $C_p \ll 1$ will be used throughout the present analysis

Primary signal to noise ratio The recording plane areas corresponding to object regions 1 and 2 are passed by N_{p1} and N_{p2} photons respectively The information relating to the difference in object transmittance is given by the difference

$$\delta N_p \equiv N_{p1} - N_{p2} \qquad (9)$$

which is thus the signal to be recorded In an actual recording experiment the value of δN_p is not a priori predictable δN_p being a stochastic variable Thus the experiment will not generally yield the mean value $\langle \delta N_p \rangle$ but some more or less deviating value The more the value actually recorded is expected to deviate from $\langle \delta N_p \rangle$ the less significance will have to be ascribed to the recorded value A measure of the expected deviation the noise of the recording is the standard deviation $\sigma(\delta N_p)$ The significance of the recording is suitably described by a signal to noise ratio defined for the primary radiation field as

$$S_p \equiv \frac{|\langle \delta N_p \rangle|}{\sigma(\delta N_p)} \qquad (10)$$

From eqs (A 11) (9) (5) and (1) it follows that

$$\langle \delta N_p \rangle = \langle N_{p1} \rangle - \langle N_{p2} \rangle$$
$$= (\Phi_{p1} - \Phi_{p2}) A = C_p \Phi_p A \qquad (11)$$

whereas (A3) (A13) (6) and (2) yield

$$\sigma(\delta N_p) = \sqrt{\sigma^2(N_{p1}) + \sigma^2(N_{p2})}$$
$$= \sqrt{(\Phi_{p1} + \Phi_{p2}) A} = \sqrt{2 \Phi_p A} \qquad (12)$$

Then the primary signal to noise ratio obtains as

$$S_p = C_p \sqrt{\tfrac{1}{2} \Phi_p A} \qquad (13)$$

which expresses the fundamental intrinsic noisiness of the primary radiation relief No actual recording process can yield a higher value for the signal to noise ratio than S_p of eq (13) Most recording processes of a photographic type a[...] from this ideal limiting case

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Still there exists a finite probability for the [...] contrast to remain undetected or be de[...] erroneously so that a dark object detail ap[...] bright in the image Suppose that $\Phi_{p1} > \Phi_{p2}$ [...] actual recording case due to quantum fluctu[...] $N_{p1} \leq N_{p2}$ may be observed This implies [...] $\delta N_p \leq 0$ or $|\delta N_p - \langle \delta N_p \rangle| \geq \langle \delta N_p \rangle$ From Čebyšev [...] equality (A 4) it follows that

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where ψ is defined by eq (3) The signal [...] $\delta N \equiv N_1 - N_2$ Application of eqs (A 11) (5) a[...] gives

$$\langle \delta N \rangle = \langle N_{p1} \rangle + \langle N_{s1} \rangle - \langle N_{p2} \rangle - \langle N_{s2} \rangle$$
$$= \langle N_{p1} \rangle - \langle N_{p2} \rangle = (\Phi_{p1} - \Phi_{p2}) A$$
$$= C_p \Phi_p A$$

the same expression as eq (11) for primary radiation alone For the variance of δN eqs (A 13) (8) and (14) yield

$$\mathrm{var}(\delta N)=\mathrm{var}(N_{pt})+\mathrm{var}(N_{s})+\mathrm{var}(N_{p})+\mathrm{var}(N_{s}) =\langle N_{pt}\rangle+\langle N_{p}\rangle+2\langle N_{s}\rangle$$

by the application of eqs (2) and (3)

$$\sigma(\delta N)=\sqrt{2(\Phi_p+\Phi_s)A}=\sqrt{2\Phi_p A(1+\psi)} \quad (16)$$

comparison with the case of no secondary radiation described by eq (12) noise has been increased a factor $\sqrt{1+\psi}$

The signal to noise ratio as defined by (10) is w extended to the case of secondary radiation from (1) (15) and (16) it is found that

$$S_t=C_t\sqrt{\tfrac{1}{2}\Phi_p A/(1+\psi)} \quad (17)$$

the signal to noise ratio has deteriorated by a factor $1/\sqrt{1+\psi}$ due to the presence of secondary radiation Contrary to commonly held views (e g EISS) the information thus lost cannot be recovered by any post recording contrast-enhancing procedure the loss is irreversible

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A secondary screening device e g a scattered ray grid is inserted into the modulated radiation field behind the object in order to reduce the relative fluence of secondary radiation (cf STRID) The reduction from the value ψ to the value ψ' is described by the device s selectivity

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$$S_t'=C_p\sqrt{\tfrac{1}{2}T_p\Phi_p A}/\sqrt{1+\psi/\Sigma} \quad (20)$$

Suppose that in a severe case e g abdominal radiography $\psi\approx7$ (WILSEY 1921) Then with no secondary screening $S_t\approx S_p/\sqrt{8}\approx0.35S_p$ An efficient optimised grid will perform $\Sigma\approx15$ with $T_p\approx0.50$ (STRID) Insertion of such a grid will improve the signal to noise ratio to $S_t'\approx0.58S_p$ as is seen from eq (20) The improvement may appear marginal However when the characteristics of the recording devices commonly used are taken into account the efficient grid will come out still more favourable

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Dynamically limited detectors The expressions (17) and (20) for the signal to-noise ratio contain the primary fluence Φ_p Only if the recording device is free from dynamic limitations can the signal to noise ratio values be compared for the same value of Φ_p Contrary to this detectors commonly used e g film screen combinations or television cameras are dynamically limited Thus a certain threshold fluence is required for a response to be produced and fluence in excess of a certain saturation value will not produce any further response The radiation relief with all its modulation detail must thus be contained within the dynamic limits of the recording device

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$$S_p=C_p\sqrt{\tfrac{1}{2}\Phi_D A}$$

$$S_t=C_p\sqrt{\tfrac{1}{2}\Phi_D A/(1+\psi)}/\sqrt{1+\psi}$$

and consequently

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$$S_t/S_p=\frac{1}{1+\psi} \qquad (21)$$

It is seen by the application of eqs (17) and (20) that the case of $\psi \approx 7$ yields $S_t \approx 0.13 S_p$ whereas the efficient grid gives $S_t \approx 0.48 S_t$ i.e. a nearly four fold improvement of the signal to noise ratio.

Comparison of different combinations of recording and secondary screening devices. Two detectors 1 and 2 requiring for optimum response fluence values Φ_{D1} and Φ_{Dr} respectively are considered. They are provided with screening devices (grids) having selectivity values Σ_i and primary transmittance values T_{pi} $(i=1, 2)$. The signal to noise ratio values should then be compared for

$$T_i \Phi_i (1+\psi/\Sigma_i) = \Phi_{Di} \tag{22}$$

For a non ideal recording device it must be taken into account that a certain fraction of the roentgen photons will be lost in the detection process. This is described by the device's detective quantum efficiency (DQE) ε being the probability for a photon to be detected. The detection loss will impair the signal to noise ratio by a factor $\sqrt{\varepsilon}$ (cf. DAINTY & SHAW 1974, § 5.1).

The mean number of photons detected by the recording device is now

$$(\delta N_i) = C_p \varepsilon T_i \Phi_i A \tag{23}$$

and the standard deviation of that number

$$\sigma(\delta N_i) = \sqrt{2\varepsilon T_{pi} \Phi_{pi} A (1+\psi/\Sigma_i)} \tag{24}$$

which is realised by the appropriate extension of eqs (15) and (16). Combination of eqs (22), (23) and (24) yields the detection signal to noise ratio

$$S_i = C_p \sqrt{\varepsilon \Phi_{Di} A} / (1+\psi/\Sigma_i) \tag{25}$$

Thus

$$S_t / S_1 = \sqrt{\varepsilon / \varepsilon_1} \times \sqrt{\Phi_{Dr}/\Phi_{D1}} \times (1+\psi/\Sigma_1)/(1+\psi/\Sigma_i) \tag{26}$$

Dose to the object. For a proper comparison between radioscopic systems to be performed the dose absorbed by the object must be considered. As far as monoenergetic radiation is concerned the dose is proportional to the incident fluence $D_i \propto \Phi_{oi} = \Phi_i / p_p$. From eq. (22) then

$$\frac{D}{D_1} = \frac{\Phi_t \, T_{p1}(1+\psi/\Sigma_1)}{\Phi_{t1} T_i \, (1+\psi/\Sigma_i)} \tag{27}$$

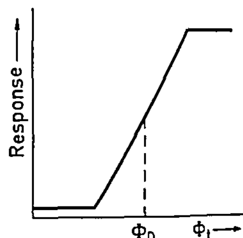


Fig. 3. Idealised characteristic of a dynamically lim... re-cording device

Comparison at fixed signal to noise ratio. Let the sensitivity values of the recording devices ... adjusted so that the two systems to be comp... yield the same value of S_t. Then eq. (26) can b... solved for the expression $(1+\psi/\Sigma_1)/(1+\psi/\Sigma_i)$ wh... in turn is inserted into eq. (27) in order to obtain

$$(D/D_1)_s = \sqrt{\varepsilon_1/\varepsilon} \times \sqrt{\Phi_{Dr}/\Phi_{D1}} \times T_{p1}/T_r \quad (\ldots$$

where the Φ_{Di} denote the sensitivity values of t... recording devices. For grids of good qua... $T_{p1} \approx T_t$ and it may as a first approximati... reasonable to assume that $\varepsilon_1 \approx \varepsilon$. The dose n th... proportional to the square root of the opt... fluence required by the detector (or loosely spe... ing inversely proportional to the square root of ... speed of the detector). It is thus justified to prop... the use of film screen combinations of ... sensitivity provided that secondary screen... be further improved (REICHMANN & STRID).

Comparison at fixed dose. Assume instead th... the systems of recording and secondary scre... devices are composed in such a way th... detectors yield optimum responses for the s... value of incident fluence. The systems can then... compared for the same object dose value. Eq. ... is solved for $(1+\psi/\Sigma_1)/(1+\psi/\Sigma_2)$ which wit... (25) yields

$$(S_t / S_{t1})_D = \sqrt{\varepsilon / \varepsilon_1} \times \sqrt{\Phi_{t1}/\Phi_t} \times \sqrt{T_t / T_1} \quad (\ldots$$

Again it may be assumed that $\varepsilon_2 \approx \varepsilon_1$ and $T_t \approx$ ... It is then found that the signal to-noise ratio f... given dose value is proportional to the square r...

he detector speed as far as improved secondary eening will admit which is consistent with the ervations by REICHMANN & STRID

Relation between contrast resolution and spatial resolution

The flux of roentgen photons being a Poisson tributed stochastic process the signal to noise io of the detection of a particular object detail determined by the number of primary photons ually penetrating that detail Thus for a given lue of the photon fluence the ratio depends on size of the projected area of the detail as shown eq (13) For an object of characteristic lateral tent d the analysis should then pertain to an mentary image area of size $A=d^2$ whence

$$S_p=C_p\, d\sqrt{\tfrac{1}{2}\Phi_p} \qquad (30)$$

d for the general case with secondary radiation d a non ideal recording device

$$S_t=C_p\, d\sqrt{\tfrac{1}{2}\varepsilon\Phi_D}/(1+\psi/\Sigma) \qquad (31)$$

s seen from eqs (13) and (25) respectively

For detection of the characteristic object detail to possible the signal to noise ratio must exceed me threshold value i e the detectability criterion n be written

$$S_t \geq S_{threshold} \qquad (32)$$

or the case of no secondary radiation and ideal tection $S_t=S_p$ and detection is then possible

$$C_p d \geq S_{threshold}/\sqrt{\tfrac{1}{2}\Phi_p} \qquad (33)$$

he implication of (33) is obvious in radioscopy nder fixed conditions the limit of contrast resolu on varies inversely with the required spatial reso ution Therefore radioscopy of fine object detail ving faint contrast demands high photon fluence nd consequently a high dose to the object

Yet the situation is much more unfavourable than 33) might indicate for the fine structure of the uman body extends in three dimensions and thin etails imply low contrast For a small detail of hickness d embedded in a thick body $C_p \propto d$ If d e taken to be the characteristic detail size in all three dimensions then the criterion (33) can be reformulated as

$$d\sqrt{\Phi_p} \geq K_1 S_{threshold} \qquad (34)$$

where K_1 is a constant Suppose that an object detail of size d_0 is barely visible at a certain value of Φ_p Then obviously the detection of a similar detail of size $\tfrac{1}{2}d_0$ will require Φ_p to be increased to 16 times the original value

The discussion of resolution limits can easily be extended to the case with secondary radiation and non ideal detection By the same considerations as led to eq (25) the criterion (34) will then read

$$d\sqrt{\varepsilon\Phi_D} \geq K_1 S_{threshold}(1+\psi/\Sigma) \qquad (35)$$

from which the detrimental effect of secondary radiation may be inferred

From the arguments leading to eq (27) the dose absorbed by the object is seen to be proportional to the fluence into the recording device

$$D \propto \frac{\Phi_D}{T_p(1+\psi/\Sigma)} \qquad (36)$$

Suppose that the recording speed described by Φ_D can be varied at will Then Φ_D is suitably elimi nated from (25) and (36) to yield the detectability criterion as

$$d\sqrt{\varepsilon T_p D} \geq K_2 S_{threshold}\sqrt{1+\psi/\Sigma}$$

or

$$D \geq K_3 \frac{S_{threshold}(1+\psi/\Sigma)}{d^4 \varepsilon T_p} \qquad (37)$$

K_3 being another constant This further emphasises the need for efficient secondary screening for given visibility requirements the dose will decrease when the relative fluence of secondary radiation admitted to the recording device is reduced provided that ε and T_p are not unfavourably affected by the im provement in secondary screening and that a corre spondingly faster recording device is used In more general terms the detectability criterion (37) demon strates the interplay of parameters pertaining to secondary screening (Σ T_p) and to the recording process proper (ε) As a corollary film screen com binations and scattered ray grids should always be considered together as far as recording quality is concerned (REICHMANN & STRID)

Concluding remarks

The consequences of quantum fluctuations in the radiation relief with regard to image quality are elucidated No experimental evidence has been provided but reference may be made to previous reports (SELIN et coll REICHMANN & STRID REICHMANN & ÅSTRAND)

Although dynamically limited recording devices have been considered the consequences with regard to image noise of the interaction between radiation and photographic type detectors have been left out of the discussion This aspect will be the subject of a forthcoming report (STRID & REICHMANN 1980)

Appendix

Elements of the theory of discrete stochastic variables

A few fundamental definitions and theorems pertaining to discrete stochastic variables will be presented For an exhaustive treatment including proofs of the theorems the reader is referred to any standard text on probability theory (e g CRAMÉR 1954 TUCKER 1962)

Mean value variance and standard deviation Any quantity ξ that can be observed in an experiment is said to be a stochastic variable Each time the experiment is performed a value for ξ is obtained A variable that takes only non negative integer values is termed discrete Let $P(\xi=k)$ be the probability that the experiment yields $\xi=k$ Then $\Sigma_{k=0}^{\infty} P(\xi=k)=1$ For many purposes the stochastic variable is adequately described by its mean value (expectation value)

$$\langle\xi\rangle \equiv \Sigma_{k=0}^{\infty} P(\xi=k)\,k \qquad \text{(A 1)}$$

and its variance

$$\mathrm{var}(\xi) \equiv \Sigma_{k=0}^{\infty} P(\xi=k)\,(k-\langle\xi\rangle) \qquad \text{(A 2)}$$

It is convenient to define the standard deviation (root mean-square deviation) of the variable as

$$\sigma(\xi) \equiv \sqrt{\mathrm{var}(\xi)} \qquad \text{(A 3)}$$

Čebyšev s inequality Probability theory provides an important theorem relating to the expected deviation of the value of ξ observed in a realisation of the experiment from the mean value

$$P(|\xi-\langle\xi\rangle| \geq a) < \mathrm{var}(\xi)/a^2 \qquad \text{(A 4)}$$

(Čebyšev s inequality) where $P(|\xi-\langle\xi\rangle| \geq a)$ denotes the probability that ξ deviates from $\langle\xi\rangle$ by at least the amount a

Sample mean value and variance Suppose that n observations of ξ yield the values ξ_1 ξ_2 ξ Then

$$\bar{\xi} \equiv \frac{1}{n} \Sigma_{k=1} \xi_k$$

and

$$s^2(\xi) \equiv \frac{1}{n-1} \Sigma_{k=1} (\xi_k-\bar{\xi})^2$$

are the sample mean value and the sample v[...] respectively of this series of observations A cor[...] a central theorem of mathematical statistics for a l[...] number of observations these quantities converge t[...] the corresponding quantities of the stochastic v[...] proper

$$\lim_{n\to\infty} \bar{\xi} = \langle\xi\rangle$$

$$\lim_{n\to\infty} s^2(\xi) = \mathrm{var}(\xi)$$

The product of a stochastic variable by a cons[...] ξ is a stochastic variable then multiplication of ξ b[...] arbitrary constant a yields a new stochastic va[...] $a\xi$ From definitions (A 1) and (A 2) it follows that

$$\langle a\xi\rangle = a\langle\xi\rangle$$

and

$$\mathrm{var}(\xi) = a^2\,\mathrm{var}(\xi)$$

The sum of or difference between two var[...] Suppose that the sum $\xi+\eta$ of two commens[...] stochastic variables ξ and η or the difference be[...] them $\xi-\eta$ is observed in an experiment Prob[...] theory relates the mean value and the variance of [...] sum or difference to the corresponding quantities of [...] constituent variables

$$\langle\xi\pm\eta\rangle = \langle\xi\rangle \pm \langle\eta\rangle$$

$$\mathrm{var}(\xi\pm\eta) = \mathrm{var}(\xi) + \mathrm{var}(\eta) \pm 2\,\mathrm{cov}(\xi\ \eta)$$

were

$$\mathrm{cov}(\xi\ \eta) \equiv \Sigma_j^{\infty} \Sigma_k^{\infty} P(\xi=j\ \eta=k)(j-\langle\xi\rangle)(k-\langle\eta\rangle)$$

is the covariance of ξ and η $P(\xi=j\ \eta=k)$ being the p[...] bility that in the same observation $\xi=j$ and $\eta=k$ [...] and η are stochastically independent i e if their [...] observed values are uncorrelated then $\mathrm{cov}(\xi\ \eta)=0$ [...] eq (A 12) simplifies into

$$\mathrm{var}(\xi\pm\eta) = \mathrm{var}(\xi) + \mathrm{var}(\eta)$$

The binomial distribution Consider a flux of ne[...] photons incident on a material body of finite th[...] Suppose that the probability for one photon t[...] the body undisturbed is p_e Then the probability [...]

oton is absorbed or scattered will be $1-p$ Transmis in of photons through a body is one example of experi nts that may yield two mutually exclusive results Suppose that the experiment is repeated N times (i e photons are sent through the body) and that N_p of se are transmitted undisturbed From combinatorics d probability theory the probability that exactly n them are transmitted will obtain as

$$P(N_p=n)=\binom{N}{n}p_p(1-p_p)^N \quad (A\ 14)$$

where $\binom{N}{n}=N!/n!(N-n)!$ This is the frequency ex ession of the binomial (Bernoulli) distribution Further lculations will show that for this kind of distribution

$$\langle N_p\rangle=Np_p \quad (A\ 15)$$

nd

$$\mathrm{var}(N_p)=Np_p(1-p_p) \quad (A\ 16)$$

The Poisson distribution The emission of roentgen hotons by the tube target may be regarded as a binomi ly distributed stochastic process Indeed during a short terval of time Δt emission may or may not occur and Δt is chosen to be sufficiently small the probability of nission of more than one photon will vanish Let $\lambda\Delta t$ e the probability for emission to take place during Δt nd let the process be observed a long time $t_{obs}=N\Delta t$ e let the experiment be performed N times During the me of observation N photons are emitted In the limit here $\Delta t\to 0$ and $N\to\infty$ in such a way that $N\Delta t$=constant he probability that $N=n$ is calculated as

$$P(N=n)=\exp(-\lambda t_{obs})(\lambda t_{obs})^n/n! \quad (A\ 17)$$

hich is the frequency function of the Poisson distribu on This distribution thus prevails as a limiting case of he binomial distribution when the number of experi nents is made very large whereas the probability of a articular result becomes infinitely small The mean value nd the variance are found to have the same value viz

$$\langle N\rangle=\mathrm{var}(N)=\lambda t_{obs} \quad (A\ 18)$$

Consider again the transmission of radiation through a material body of finite thickness Let the flux density be the area irradiated A and the observation time t_{obs} Then on an average the number of incident photons is $\langle N_0\rangle=\varphi At_{obs}$ Emission of roentgen photons being a Poisson-distributed process the standard deviation of N_0 is $\sigma(N_0)=\sqrt{\varphi At_{obs}}$ Inside the body photons will be absorbed or scattered leaving the probability p_p for a photon to be transmitted undisturbed If the number of incident photons in an actual experiment were n_0 then qs (A 15) and (A 16) would yield for the number of photons transmitted $\langle N_p\rangle=n_0p_p$ and $\mathrm{var}(N_p)=n_0p_p(1-p_p)$ However the actual number of incident photons is deter mined by the stochastic variable N_0 and so the actual distribution of N will prevail as an average for all possible values of n_0 Straightforward calculation will yield

$$\langle N_p\rangle=\mathrm{var}(N_p)=N_0p_p \quad (A\ 19)$$

for a Poisson-distributed flux of incident photons the flux of transmitted photons is Poisson distributed as well

The flux of secondary (i e scattered and fluorescent) photons may be treated analogously leading to the same conclusion

SUMMARY

Quantum fluctuations in the roentgen radiation relief are analysed mathematically The intrinsic signal to-noise ratio of the radiation relief for a given object contrast is proportional to the square root of the number of photons contributing to the image of a characteristic detail in the object In the presence of secondary radiation the signal to noise ratio is impaired since the fluctuations of secondary radiation increase the noise of the radiation relief By efficient secondary screening the quality of the relief can be partially recovered With a dynamically limited recording device i e a film-screen combination increased detection speed in conjunction with improved secondary screening will either result in unchanged image quality with the gain of an object-dose reduction or provide improved imaging at an unchanged dose value As regards the relation between contrast resolution and spatial resolution the dose required to barely demonstrate an object detail of given geometry and composition is found to vary inversely with the fourth power of the linear size of the detail It is also concluded that recording and secondary screening devices should always be considered together as far as recording quality is concerned

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M RADIUMHEMMET KAROLINSKA SJUKHUSET S 104 01 STOCKHOLM AND THE CENTRAL MICROBIOLOGI CAL LABORATORY OF STOCKHOLM COUNTY COUNCIL S-101 22 STOCKHOLM SWEDEN

NATURAL KILLER ACTIVITY IN PERIPHERAL LYMPHOCYTE POPULATION FOLLOWING LOCAL RADIATION THERAPY

H Blomgren E Baral F Edsmyr L E Strender
B Petrini and J Wasserman

Lymphocytes which express natural cytotoxicity r tumour cells in vitro have recently received ich interest because of their possible role in sur illance mechanisms A positive correlation has en found in experimental animals between natural ller (NK) activity in vitro and capacity to prevent owth of transplanted tumours (Haller et coll 977 Kasai et coll 1979 Kiessling et coll 1975 'Arner et coll 1977) Moreover mouse strains isplaying a high incidence of spontaneous leukemia xhibit low NK activity in vitro and vice versa Zarling et coll 1975)

Although the role of NK cells in tumour cell sur eillance in the human is unknown it is of interest to xamine whether they are affected by treatments hich are frequently used to control human malig ant tumours In the present investigation the NK ctivity in peripheral lymphocytes has been xamined after postoperative radiation therapy for reast carcinoma Previously it has been shown that his treatment induces lymphopenia with an altera ion in the proportion of lymphocyte subpopulations Petrini et coll 1977) reduces the responses of the ymphocytes to soluble antigen and allogeneic cells Baral et coll 1977 Blomgren et coll 1977) and educes the capacity of the cell population to nediate antibody dependent cellular cytotoxicity ADCC Wasserman et coll 1975)

Material and Methods

The material consisted of 24 women with primary breast carcinoma (32–70 years old) 6 patients with carcinoma of the prostate (62–77 years old) and 7 patients with carcinoma of the urinary bladder (1 female and 6 males with an age range of 61–77 years) The patients with breast carcinoma were considered operable at diagnosis but not the patients with carcinoma of the prostate or of the bladder

Irradiation techniques Patients with breast car cinoma were treated with a modified radical mastec tomy followed by local irradiation up to a total target dose of 45 0 Gy (4500 rad) as described by Ide strom et coll (1979) Patients with carcinoma of the prostate received local irradiation of the tumour covering the entire small pelvic region but not ex tending to the juxta regional nodes The calculated mean tumour dose of 54 0 Gy was given in 6 weeks Patients with carcinoma of the bladder received ir radiation of the bladder with a wide margin including the regional lymph nodes in the 75 per cent isodose curve The calculated total tumour dose was 64.5 Gy given during 8 weeks with a break of 2 weeks after 32 0 Gy Patients with carcinoma of the prostate or

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bladder were treated with 6 MV roentgen rays using a 3 field technique with 2 wedge filter beams in the front and one open beam from the back.

Blood sampling. Blood samples were obtained from the patients with breast carcinoma at four occasions: sample number I within one week before postoperative irradiation was started, sample II within one week after completion of irradiation, sample III three to four months after irradiation and sample IV six to eight months after irradiation. Two blood samples were obtained from the patients with carcinoma of the urinary bladder or the prostate within one week before start of irradiation and within one week after a total target dose of 54.0 Gy (carcinoma of the prostate) and 64.5 Gy (carcinoma of the urinary bladder).

Separation of lymphocytes. Lymphoid cells were separated from heparinized venous blood by centrifugation on Ficoll Isopaque and phagocytic cells were removed magnetically (BLOMGREN 1974). The resultant preparations were used for testing the NK activity of the lymphocyte preparation. T-cells were separated from these purified lymphocyte preparations by rosetting with neuraminidase treated sheep red blood cells (E_N) followed by centrifugation on Ficoll Isopaque (MORETTA et coll. 1977).

Rosette techniques. Details of these techniques have been presented previously (PETRINI et coll. 1979). Lymphocytes possessing membrane receptors for the Fc part of IgG were identified in non-separated preparations by their capacity to form rosettes with ox RBC sensitized with rabbit anti ox RBC IgG. Lymphocytes in T-cell enriched fractions possessing Fc receptors for IgG (T_G-cells) or IgM (T_M-cells) were identified by their capacity to form rosettes with ox RBC IgG and IgM, respectively. The frequency of T-cells was established by counting cells forming rosettes with E_N.

Cytotoxic test. NK activity was measured by incubating lymphocytes for 4 h with ^{51}Cr labelled target cells termed K562 (derived from a human myeloid leukemia) and Chang cells (probably derived from human liver) as described previously (EINHORN et coll. 1978). A cytotoxic index was calculated according to the following formula: (% release with lymphocytes–% spontaneous release)/(100–% spontaneous release).

Duplicate tests were performed and variability within the duplicates did not exceed 10 per cent

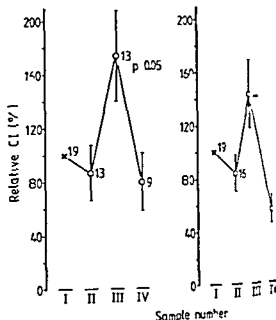


Fig. 1. Relative changes of the NK activity expressed as cytotoxic index (C.I.) of the peripheral lymphocyte preparations against Chang cells after irradiation for mammary carcinoma. Left diagram shows the results employing a lymphocyte: target cell ratio of 50:1 and the right diagram 100:1. Pretreatment values are set as 100 per cent. The absolute pretreatment cytotoxic indices (mean ± SE) were 0.16±0.04 and 0. ±0.04 respectively. Mean values ± SE are shown and figures at the symbols indicate the number of patients. A p-value at a symbol indicates that the mean differs significantly from the pretreatment value.

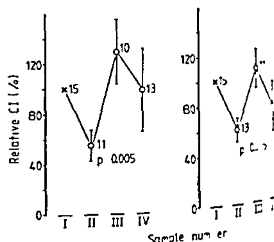


Fig. 2. Same as in Fig. 1 except that K562 cells were used as target cells. The absolute pretreatment cytotoxic indices (mean ± SE) were 0.54±0.05 using a lymphocyte: target cell ratio of 50:1 and 0.63±0.04 using a ratio of 100:1.

tient. The Student's t test was used for calculating whether the changes were statistically significant.

Results

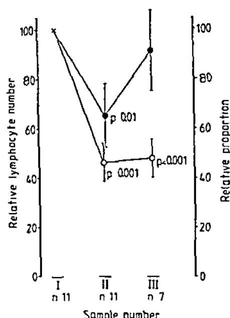


Fig. 3 Relative lymphocyte counts and relative proportions of lymphocytes possessing Fc IgG receptors in non fractionated cell preparations after irradiation of mammary carcinoma. The pretreatment values are set as 100 per cent. The absolute pretreatment lymphocyte count (mean ± SE) was 2000± 00 and the percentage of Fc IgG bearing cells 19 7±? 4. Mean values ± SE are shown. A p value at a symbol indicates that this mean value differs significantly from the pretreatment value. Relative lymphocyte counts (O), relative proportion of lymphocytes possessing Fc IgG receptors (●)

Table

Percentage of lymphocytes forming rosettes with E_A in non fractionated lymphocyte preparations and the frequency of lymphocytes possessing Fc receptors for IgG (T_G) and IgM (T_M) in E_A rosette sedimented fractions. The determinations were made in patients before irradiation and at completion of pelvic irradiation. Mean values ± SE are shown

	Before irradiation	After irradiation
binding cells	79 9±? 0	73 .± 3 5 (9 2± 4 8)
-cells	. 5±1 6	.4 6± 5 0 (115 0± 3 1)
-cells	37 3±3 5	39 9± 6 6 (110 8±16 0)

Mean values ± SE expressed as per cent of the pretreatment values. No statistically significant changes after irradiation

activity of the lymphocyte population exhibited a small but non significant reduction for Chang cells at completion of radiation therapy for breast carcinoma (Fig 1). This was followed by a significant over shoot three to four months later employing a lymphocyte target cell ratio of 50 1. Six to eight months after irradiation (sample 4) NK activity was reduced to the original level or below this level. Most of the patients in this group were also examined for NK activity against K562 cells (Fig 2). The NK activity against this target cell largely changed similar to that recorded against Chang cells with the exception that the initial reduction was statistically significant and the ensuing increase less marked.

Frequency of Fc receptor bearing lymphocytes following irradiation. Since lymphocytes mediating NK activity in the human seem to possess Fc receptors for IgG (COOPER et coll 1977) the proportion of such cells following irradiation was examined. The changes of the lymphocyte counts and the relative proportion of lymphocytes possessing Fc receptors for IgG following irradiation for breast carcinoma appear in Fig 3. Five of the patients included in this group were also tested for NK activity. The lymphocyte counts were reduced to approximately 45 per cent and the relative proportion of Fc IgG positive cells was reduced to approximately 65 per cent at completion of irradiation. Three to four months after treatment (sample 3) the lymphocyte counts remained at essentially the same level whereas the frequency of Fc IgG positive cells had recovered to approximately 90 per cent of the original value.

Since the NK cell is considered to be a non T-cell by COOPER et coll and since the T-cell population in the blood also contains Fc IgG receptor positive cells (T_G) it was of interest to know whether the decline of the proportion of Fc receptor bearing cells was due to a reduced frequency within the T or the non T lymphocyte compartments. This was analysed in a group of patients who received radiation therapy to the pelvic region and whose lymphocyte counts were reduced to 40 to 60 per cent of the pretreatment values. The frequency of lymphocytes forming E_A rosettes did not change (Table) and the frequency of T_G cells within this cell population was not altered significantly. The proportion of T_M cells also remained essentially constant.

Discussion

The NK activity of the peripheral lymphocyte population was analysed on a cell for-cell basis before and after postoperative radiation therapy for breast carcinoma. Such knowledge may be relevant since data from animal experiments indicate a close

correlation between in vitro NK activity and growth of tumour cells in vivo

Somewhat different results were obtained depending on the type of target cell and the lymphocyte target cell ratio NK activity against K562 cells was significantly reduced shortly after completion of irradiation whereas such a reduction was hardly discernible against Chang cells (Figs 1 2) Three to four months after irradiation the NK activity against Chang cells was above the pretreatment level followed by a decline No significant overshoot was observed using K562 cells as targets These results may indicate that the lymphocyte population mediating NK activity is heterogeneous with respect to radiation sensitivity and mode of recovery after irradiation Moreover as shown in Figs 1 and 2 the amplitudes of the changes of the NK activity after irradiation may be dependent on the lymphocyte target cell ratio

The frequency of lymphocytes possessing Fc receptors for IgG in non fractionated lymphocyte preparations varied similarly to that of the NK activity against K562 cells (Fig 3) These lymphocytes may be mainly non T-cells as the frequency of Fc IgG receptor bearing lymphocytes within the T-cell population was not significantly changed in patients irradiated for pelvic tumours (Table) Provided that these data can be applied to patients with breast carcinoma it is possible that the fluctuations of the relative NK activity may at least partly be due to a changed frequency of Fc IgG bearing non T-cells

It has been observed that local radiation therapy for pulmonary carcinoma augments the cytotoxicity of the peripheral lymphocytes against allogeneic pulmonary carcinoma cells as measured one week after completion of therapy (MANABE et coll 1977) Radiation therapy for ovarian carcinoma stage III did not change the cytotoxicity of the peripheral lymphocytes against allogeneic ovarian carcinoma cells in spite of a profound radiation induced lymphopenia In contrast radiation therapy for ovarian carcinoma stage I and II caused a significant reduction of cytotoxicity after seven days of treatment which however recovered during continuation of treatment (KOHORN et coll 1978) A reduction of cytotoxicity of peripheral lymphocytes against allogeneic urinary bladder carcinoma cells after irradiation was observed by O TOOLE et coll (1973)

It should be emphasized that in the investigations mentioned the target cells for cytotoxicity were of the same histologic type as the patient tumours and probably partially of ADCC type Therefore the results may not be strictly comparable with those of the present report However several experiments performed in animals have shown that the NK cells are relatively resistant to radiation as measured within 24 h of irradiation (HOCHMAN & CUDKOWICZ 1977 KIESSLING et coll 1977 OEHLER & HERBERMAN 1978) HOCHMAN et coll (1978) observed that NK activity in the spleen did not decline until [illegible] days after whole body exposure of mice to [illegible] Gy (originally 700 R) and recovery started on day [illegible] They concluded that mature NK cells are relatively radiation resistant whereas their progenitors are relatively sensitive In contrast it was concluded by OEHLER & HERBERMAN that rat pre NK cells are more resistant than mature NK cells since exposure of rats to about 10 Gy (originally 1000 R) abolished the NK cell activity which however was restored by treating animals with poly I C a possible activator of pre NK cells

The fluctuations of the NK activity after radiation therapy as observed in the present investigation may indicate that mature NK cells or their progenitors or both are affected by the treatment In an attempt to elucidate this question an investigation has been started to reveal whether these fluctuations disappear by treating the lymphocytes with interferon which is known to augment NK activity (EINHORN et coll TRINCHIERI & SANTOLI 1978) Also such tests may also reveal whether the NK activity of patient lymphocytes correlate to prognosis of the disease

SUMMARY

The natural killer (NK) activity of peripheral lymphocytes against ^{51}Cr labelled Chang or K562 cells was tested using a 4 h release assay before and after postoperative radiation therapy for mammary carcinoma NK activity against K562 was significantly reduced at completion of therapy (a total target dose of 45 0 Gy) and [illegible] months later NK activity against Chang cell [illegible] slight but non-significant decline at completion of [illegible] followed by an overshoot 3 to 4 months later The frequency of Fc IgG receptor bearing lymphocytes [illegible] creased at completion of therapy and largely [illegible] 3 to 4 months

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EFFECT OF DIFFERENT ^{90}Sr DOSES ON THE MICROSCOPIC STRUCTURE OF FOETAL MOUSE OVARIES

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Radioactive isotopes accidentally incorporated ito females during pregnancy will consequently so place their foetuses at risk of radiation injury his might disturb the fertility conditions of the con iminated young and even lead to sterility The ra iation sensitivity of the female gonads have there ore been investigated which revealed great varia ons depending on the stage of development during xposure (PETERS & LEVY 1964 OAKBERG 1968 ÖNNBACK et coll 1971) An internal ^{90}Sr contami ation was shown to injure the foetal ovaries more verely than did acute roentgen irradiation with 19 and 0 76 Gy (20 and 80 R respectively HEN ICSON & NILSSON 1970) The effect on the ovaries f in utero ^{90}Sr treated mouse foetuses depends con iderably on the time for contamination during the estation (RÖNNBACK 1979) The most severe ef cts were observed when the contamination oc urred during the last part of the pregnancy

The injury to foetal ovaries identified at micro copy was therefore correlated to different amounts f ^{90}Sr injected into pregnant mice on the 19th day ost coitum The results are now presented

Material and Method

Female CBA mice aged 70 to 75 days were nated to untreated males of the same strain The females were controlled daily and the presence of a vaginal plug indicated the onset of pregnancy (the day for vaginal plug was called day 1) On day 19 post coitum the pregnant females were intravenous ly injected into a tail vein with a ^{90}Sr solution to give an amount of 11 1 to 370 kBq (0 3–10 0 μCi) per pregnant female in different experiment groups The treated females were compared with mated un treated control females The nuclide was adminis tered as carrier free ^{90}Sr nitrate in 1 N nitric acid diluted with physiologic saline to give a strength of about 2 6×10^6 Bq/ml (70 μCi/ml) When injected the solution was in equilibrium concerning ^{90}Sr and ^{90}Y (The strontium solution was supplied by the Radio chemical Centre Amersham England)

The experiment schedule gives the strontium doses administered to the pregnant females in differ ent groups and the number of randomly selected in utero treated females which were killed at day 28 56 or 84 post partum for analysis of their ovarian content

Two experiment series with about half a year in between were performed A control series was run in each part The higher dose groups 46 3 to 370 kBq (1 25–10 μCi) were run first though the presenta tion will be given with increasing ^{90}Sr-dose (cf schedule) The dose of 46 3 kBq was run in both parts to enable a comparison

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Experiment schedule

Dose (kBq) per pregnant female		No. of in utero treated females analysed in different groups		
		Time for killing (days p.p.)		
		28	56	84
Control	(untreated)	5	5	–
11.1	(0.3 µCi)	5	5	–
22.2	(0.6)	5	5	5
46.3	(1.25)	4	5	5
Control	(untreated)	4	4	5
46.3	(1.25 µCi)	4	5	5
92.5	(2.5)	5	5	5
185	(5.0)	5	5	5
370	(10.0)	5	5	5

The asterisks indicate with which control group the 46.3 kBq group in question should be compared.

After killing the females, which as far as possible did not originate from the same litter, their ovaries were prepared and immediately fixed in Stieve's fluid. They were treated according to conventional histologic methods and embedded in Paraplast. The left ovary from every female was serially sectioned with a thickness of 5 µm and every 10th section was brought to microscopic analysis including counting oocytes and follicles in different stages of development.

The cell material was divided into 7 classes as in previous investigations: oocytes of type I without any surrounding granulosa cells, type II and III oocytes with an increasing number of such cells (but not forming a complete layer), primary folicles with one complete layer of granulosa cells and growing follicles with several layers but with no antrum formation, early antrum follicles with the antrum initiated and finally Graafian follicles with a complete antrum formation in the follicular epithelium. Two degenerating stages were also counted: atretic follicles and corpora atretica. The former with lytic and pycnotic cells in the follicular layers and often with a shrunken oocyte and the latter being the final stage of atresia in growing and Graafian follicles.

The observed number of cells in these nine stages was adjusted to give the total number in the ovary by means of a correction formula (ABERCROMBIE 1946). These corrected figures from the strontium groups were compared with those of corresponding controls to give an estimation of the effect in terms of a reduced number of cells caused by the nuclide.

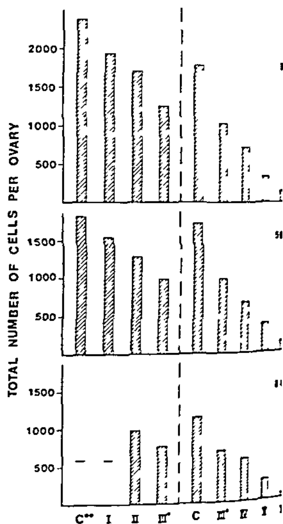
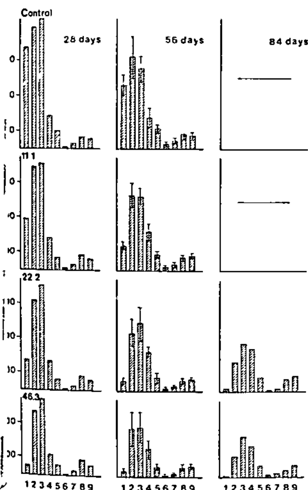
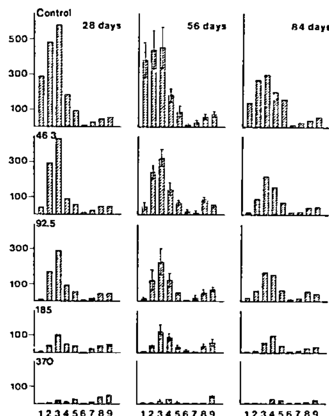


Fig. 1. Total number of remaining cells in ovaries fro[m] ... treated with ⁹⁰Sr on day 19 [of] their foetal ... females were examined when aged 28, 56 or 84 da[ys] ... untreated control (second part), I: 11.1 kBq, II: ... III: 46.3 kBq per pregnant female, respectively, C: ... (first part), III: 46.3 kBq, IV: 92.5 kBq, V: 185 kBq, VI: ... per pregnant female, respectively. The asterisks ... which control group the 46.3 kBq group in question sho[uld be] compared.

Results

The total number of cells, calculated on the ... ovary, is given in Fig. 1. Every comparison be[tween] strontium treated groups and correspond[ing] ... groups reveals a statistically significant de[crease in] the number of cells. Fig. 2 presents in the sa[me way] the number of oocytes and follicles in th[e] ... of development in the two control gro[ups] as w[ell as] in the strontium treated groups. The am[ount] ... between 11.1 and 320 kBq per animal ... graphs also show the variation in the number of ... by age, as the figures are given from anim[als] ... 28, 56 or 84 days post partum. (In the second ... no analyses were available from animal ... day 84 in the control and 11.1 kBq groups.)





g 7 The number of cells in different stages of development (1–9) for ⁹⁰Sr doses between 11 1 kBq and 370 kBq given on day 19 post coitum Stage 1 oocyte type I 2 oocyte type II 3 oocyte type III 4 primary follicles 5 growing follicles 6 early antrum follicles 7 Graafian follicles 8 atretic follicles 9 corpora atretica

The frequency of oocytes I–III at day 84 was about half of that at day 28 irrespective whether the animals were strontium treated or not The frequencies of cells in the following stages were not remarkably altered neither in the control groups nor in the strontium treated ones

The naked oocyte (stage I) has previously been found to be very sensitive to radiation Also in the present investigation appeared a strongly significant decrease of the number (to 50 % of the control value) even in the lowest dose group where 11 1 kBq ⁹⁰Sr was administered to the investigated animals mother during her pregnancy This observation was made both at day 28 and day 56

When the dose increased to 22 2 kBq the effect was much more marked and also the stages of oocyte II and III were affected After 46 3 kBq only about 10 per cent of type I oocytes and between 50 and 60 per cent of oocytes II and III r t ine l compared with corresponding control figures i e statistically significant decreases in all three stages as well as in the number of Graafian follicles Still higher doses caused stronger effects and after 370 kBq naked oocytes and practically also type II oocytes were eliminated and only about 3 per cent of type III oocytes remained Also the other stages except for corpora atretica (stage 9) were severely affected

Within the dose range 11 1 to 185 kBq ⁹⁰Sr the primary follicles (stage 4) showed a tendency to increase in number by age a tendency not found in untreated animals

In the two lowest dose groups the number of primary follicles at day 56 had increased compared with the controls (though not statistically significant) After higher doses a reduced number of primary follicles was observed in accordance with that of the level of other cell stages

The frequencies of cells in the nine stages were

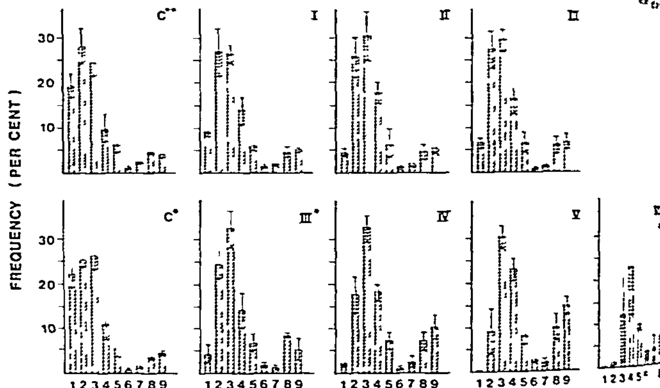


Fig. 3. The frequencies of cells in the different stages (cf. Fig. 2) calculated as per cent of the total number of cells per ovary for every individual. Mean ± deviation at a 5% risk level.

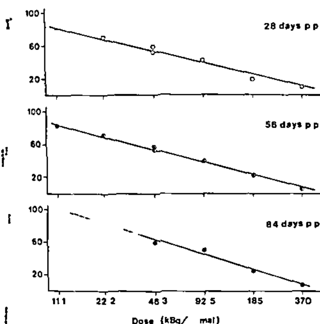
calculated as per cent of the total number per ovary for every individual in order to eliminate differences depending on variations between females in their total number of oocytes and follicles. These figures gave the means and deviations at a 5 per cent risk level at day 56 (Fig. 3). At day 28 and day 84 the animals showed a similar trend when compared with controls of corresponding age. The decrease in the number of naked oocytes is evident also when calculated in that way. That decrease is the only major change in the two lowest dose groups, but a tendency to an increased fraction of early antrum follicles might also be observed. With doses of 46.4 kBq ^{90}Sr and higher, the fraction of young oocytes still decreases. The fraction of primary follicles (stage 4) seems to increase by dose. In the highest dose groups there seems to be an increase of the fraction of degenerating stages (8 and 9) depending on that way of calculation.

The total number of cells per ovary at the three examined ages is given in Table 1. In Table 2 these figures have been recalculated to give the frequency of cells in the strontium groups expressed as per cent of those in corresponding control groups. The percental figures were then plotted against dose (i.e. the amount of strontium) in a semilogarithmic diagram (Fig. 4). At day 84 no figures were available for 11.1 and 22.2 kBq ^{90}Sr and therefore the line has been extrapolated for these doses. A ... negative correlation is evident between the ... tude of the remaining cell fraction in females ... in utero and log dose, when the nuclide was administered on the gestational day 19.

Discussion

Investigations on foetal ovaries have shown ... high sensitivity to ionizing radiation ... depending on the injury to (and thus elimination of) ... oocytes. The loss of cells is, according to the present knowledge, looked upon as irreparable ... might influence the fertility conditions and the ... reproductive capacity of such in utero ... females.

It has been shown (RÖNNBACK 1979) that ... when the contamination occurs during the ... development is a very important factor ... the degree of injury to the foetal ovaries. ... mice were internally contaminated with ... day 15 post coitum, the injury expressed as ... duction of the number of oocytes and f...



ig 4 Remaining fraction of cells in 90Sr treated ovaries (in per nt of respective controls) versus log dose

Table 1

Total number of cells per o ary (mean±SE)

	Days post partum		
	?8	56	84
ontrol	2 4?9 5±62 3	1 836 0±65 7	–
1 1 kBq	1 935 4±4? 9	1 531±40 8	–
? 2	1 707 6±48 7	1 295 7±51 1	995 6±44 9
6 3	1 254 ?±49 6	99? 6±59 2	780 1±40 9
ontrol	1 77? 9±35 2	1 723 1±76 8	1 145 4±36 5
6 3	1 033 4±45 6	991 8±36 1	685 2±36 4
? 5	735 7±28 4	685 4±45 4	586 3±22 6
85	333 3±25 5	389 ?±?? ?	?80 8±21 6
70	172 7± 8 9	134 1±1? 1	95 7±11 3

he asterisks indicate with which control group the 46 3 kBq roup question should be compared

naining in the adult females was much stronger han after a contamination during any earlier part of he pregnancy The most severe effect was found when the contamination occurred on day 19 with only about 5 per cent of the cells remaining It was proposed that this strong effect mostly was de eloped by 90Sr incorporated into the foetal skele on where it could act as a permanent radiation ou e during the radiation sensitive period follow ng birth

NILSSON & HENRICSON (1969) used 740 kBq (20 μCi) 90Sr per pregnant female RÖNNBACK (1979) 370 kBq when he analysed the correlation between the time for contamination and the injury to the ovaries and it was fully evident that these strontium doses caused serious reductions of the number of oocytes and follicles

Table 2

The frequency of cells in different dose groups given as per cent of respective control values

	Days post partum		
	28	56	84
11 1 kBq	79 7	83 4	–
?2 1	70 3	70 6	–
46 3	51 6	54 1	–
46 3	58 3	57 6	59 8
92 5	41 5	39 8	51 ?
185	18 8	22 6	?4 5
370	9 7	7 8	8 4

The purpose of the present investigation was to find out how small an amount of 90Sr administered on day 19 of foetal development that could give a microscopically notable reduction of the number of oocytes and follicles in the foetal ovaries

The experiment had to be performed in two sepa rate parts the one with higher strontium doses being run first A comparison of the figures in Table 1 from females at day 28 indicates that a (significant) differ ence exists both between the two control groups and between the 46 3 kBq groups at that age However this difference had more or less disappeared at day 56 Nor was there any significant difference at day 84 between the two 46 3 kBq groups

These discrepancies are not considered having seriously influenced the further evaluation of the experimental data as presented in Fig 4 The re maining fraction of cells in the 90Sr treated ovaries expressed as per cent of corresponding control fig ures were plotted in semilogarithmic diagrams ver sus the dose (i e the amount of 90Sr injected into the pregnant females) The plotted lines reveal that the injury (reduced number of cells) is strongly corre lated to log dose It is also evident that the rate of injury is almost identical for the ages examined as the slope of the three lines is practically identical Therefore it might be suggested that the reduction seems to occur at the same rate independent of the initial number of cells

As stated previously (RÖNNBACK et coll RÖNN

Table 3

Retention of ^{90}Sr and ^{90}Y in ovaries of 10-day-old females and their mothers when injected on day 19 post coitum. ^{90}Sr in equilibrium with ^{90}Y 370 kBq ^{90}Sr injected per pregnant female. Data from the mothers of the litters are given on the same line

Offspring							Mothers			
No of offspring		Ovaries	Total weight of ovaries (mg)	Per cent of injected amount per kg tissue		Ratio ^{90}Y/^{90}Sr	Total weight of ovaries (mg)	Per cent of injected amount per kg tissue		Ratio ^{90}Y/^{90}Sr
M	F			^{90}Sr	^{90}Y			^{90}Sr	^{90}Y	
3	5	7	7 1	139	45	0 33	13 0	8 3	57	6 8
3	5	8	18 1	267	154	0 58	11 6	3 7	31	13 4
5	2	4	6 9	24	13	0 56	17 7	20 3	41	0
4		4	11	19	8	0 41	26 2	9 9	22	2
1	8	13	17 4	50	6	0 53	14 8	44 0	86	0
8		4	5 5	57	57	1 00	17 9	3 8	3 [illegible]	1 0
7	3	4	7 8	31	18	0 57	14 5	4 5	15	3 4
Mean			1 56	83 9	45 9	0 57	8 26	13 5	39 4	4 4
±SE			±0 70 (mean weight of one ovary)	±34 16	±19 21	±0 08	±0 91 (mean weight of one ovary)	±5 54	±10 67	±1 66

BACK 1979) young oocytes and especially those in the first stage of development without any surrounding granulosa cells have an extremely high radiation sensitivity The present investigation reveals that even 11 1 kBq ^{90}Sr given to the pregnant females significantly reduced the number of oocytes in the foetal ovaries

The real numbers of remaining oocytes and follicles in the different dose groups are given in Fig 2 A similar but somewhat different way to present the effect of the nuclide appears in Fig 3 where the Y axis indicates the frequency of cells (given as per cent) in different development stages calculated on the total number of cells per female These histograms underline the fact that especially the frequencies of naked oocytes are affected by very low doses of the nuclide On the other hand the proportions of the next two stages of developing oocytes seem not to change until the strontium doses reach 92 5 kBq (2 5 μCi) and higher The increased proportion of degeneration cell stages (8 and 9) at higher doses is a consequence of the presentation technique (cf Fig 2 b where the actual numbers of these stages decrease)

It has not been possible to determine the uptake of ^{90}Sr and ^{90}Y in the foetal ovary with available technique APPELGREN et coll (1966) have shown by autoradiography that the ovaries and in particular the follicle walls of non pregnant mice have a high accumulation capacity with regard to ^{90}Y [illegible] observation has been corroborated by WALINDER & MULLER (1975) who also found that the [illegible] ^{90}Y in soft tissues was much higher than that of [illegible] when the latter nuclide was administered [illegible] rium with ^{90}Y to adult female mice In add[illegible] found that the uptake of ^{90}Sr in the mouse ovary [illegible] significantly higher than that in other soft tissues

In an attempt to achieve a crude indication [illegible] uptake and retention of ^{90}Sr and ^{90}Y in the ovaries [illegible] the foetuses and their mothers 370 kBq of ^{90}Sr [illegible] equilibrium with ^{90}Y was injected in [illegible] pregnant mice on day 19 of gestation The [illegible] and their offspring were killed when the [illegible] 10 days old The ovaries were prepared [illegible] measured with respect to their ^{90}Sr and ^{90}Y [illegible] using the methods of WALINDER & MULLER [illegible] results are given in Table 3 where the data [illegible] each litter are presented on the same line [illegible] from the mother the corresponding [illegible] non mated females are presented in Table [illegible]

The activity concentrations [illegible] the mothers as well as in their offspring [illegible] so than in the non mated mice Due to these [illegible] tions it is impossible to receive [illegible]

Table 4

etention of ^{90}Sr and ^{90}Y in ovaries of non mated females 10 ays after the i yection of a ^{90}Sr solution in equilibrium with ^{90}Y 370 kBq ^{90}Sr injected per female

	Total weight of ovaries (mg)	Ratio $^{90}Y/^{90}Sr$	Per cent of injected amount per kg tissue	
			^{90}Sr	^{90}Y
	3 0	2 7	9 5	26
	[illegible]1 6	1 8	13 1	[illegible]4
	7	2 1	5 4	11
	21 6	2 8	7 9	22
	[illegible]0 0	[illegible] 7	8 9	[illegible]4
Mean±	10 90	2 4	9 0	[illegible]1 4
E	±0 27 (mean weight of one ovary)	±0 [illegible]0	±2 [illegible]4	±2 68

nces between the concentrations of activity in the aternal ovaries and those in the offspring as well as etween mothers and non mated females There is a ossible significance (p<0 05) in the ovarian $^{90}Y/^{90}Sr$ atios between the litters and their mothers and a orresponding clear significance (p<0 001) between he young and the non mated females indicating a igher uptake of ^{90}Sr than of ^{90}Y in the foetal ovary ompared with that in the adult mouse The high ıptake and the long lasting activity in the foetal ovary imply indubitably a considerable contribution o the radiation dose to the oocytes

In addition the mineralization of the foetal skele on causes an incorporation of great amounts of ^{90}Sr and ^{90}Y in growing bones The radiation from these deposits to the ovaries is certainly not negligible The dose contributions from deposits in the mater nal skeleton is—according to autoradiographic evi dence—probably of less importance

The figures in Table 3 indicate that the radiation dose of the foetal ovary is high but even if contribu tions from ^{90}Sr and ^{90}Y deposits in the foetal skele ton are taken into account it is not likely that the doses to the foetal oocytes are much higher than doses to the oocytes in the adult mouse when in jected with the same amount of ^{90}Sr This conclusion is all the more probable as the high activity concen tration in the mature follicles has no correspondence in the foetal ovary

The marked effects on the oocytes in mice ex posed in utero to ^{90}Sr and ^{90}Y must therefore in all essentials be explained by the high radiation sen sitivity of the young oocytes especially as they have been exposed during stages of extreme suscepti bility i e just before the onset of and partly during the period of arrested development (the dichtyate stage) around the birth and the first two weeks of age (RUGH & WOHLFROMM 1964 RONNBACK 1967)

The estimation of the retention of the nuclide in the juvenile ovaries at day 10 reveals great varia tions between individuals Such variations were also found in the ovaries from the mothers as distin guished from the results in non mated females Whether these variations reflect corresponding dis parity in the radiation doses seems doubtful since the microscopic findings do not show differences of the same order of magnitude between individuals in the same dose group This might be considered as a dominance of the radiation from the foetal skeleton to the ovaries being so much higher than the con tributions from nuclides deposited in the ovarian tissues

Though there is a statistically significant loss of young oocytes in mice treated in utero with very low doses of ^{90}Sr a pool of functional follicles seems to remain Preliminary results in a fertility experiment in mice with doses similar to those given in the present one indicate that this pool during certain circumstances might be large enough to permit a nearly unchanged reproductive capacity in the adult females However it must also be remembered that the number of remaining oocytes and follicles are given per one ovary and thus must be doubled to be valid for the individual Samples taken at random from the present material have not given evidence for differences in the number of oocytes and follicles between the ovaries in the same individual as was also found in monkeys by ANDERSEN et coll (1977)

SUMMARY

Pregnant CBA mice were injected with different doses of ^{90}Sr on the 19th day post coitum The doses ranged between 11 1 and 370 kBq (0 3–10 0 μCi) per animal The ovaries of the in utero treated females were analysed when the animals were 28 56 or 84 days old respectively The effect of the nuclide was expressed as a reduction of the number (frequency) of cells in different stages of ovarian development in a comparison between ^{90}Sr treated animals and untreated controls The injury (reduced number of cells) was strongly correlated to log dose The most radia

tion sensitive stage (the naked oocytes) was clearly affected even at the lowest dose level The different ovarian ^{90}Y/^{90}Sr ratios in litters and their mothers are discussed

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CHROMOSOME COUNTS OF ^{90}Sr-INDUCED OSTEOSARCOMAS IN MICE

II. Variation of the chromosome counts of slow and fast growing tumours in hyper- and nonhyperimmunized hosts

H. BERGMAN

Osteosarcomas induced by ^{90}Sr have been examined for different characteristics previously (NILSSON 1962, 1966, 1969; NILSSON & RÖNNBÄCK 1973). Data indicating that such osteosarcomas possess specific transplantation antigens were presented by NILSSON et coll. (1972). It was then observed that the incidence of progressively growing tumours was significantly greater in whole-body irradiated (4 Gy, 400 R) than in unirradiated mice. Furthermore, heavy irradiation (150 Gy, 15 000 R) of tumour cells before transplantation decreased the frequency of progressively growing tumours in comparison to treatment with heavily irradiated normal tissues.

Chromosome number variation in serially transplanted ^{90}Sr-induced bone tumours in CBA mice has been reported by BERGMAN & NILSSON (Part I). Previous data have shown that, when relating the outgrowth period of primary tumours to the chromosome pattern (KATO 1968; MARK 1969; MITELMAN 1971), fast growing tumours exhibited a normal chromosome distribution while tumours with deviating chromosome numbers were slower growing. Therefore, a primary aim of the present investigation was to test whether a connection may exist between the outgrowth period and the variation in chromosome pattern of transplanted ^{90}Sr-induced tumours. For this purpose separate serial transplantation was performed from both a fast and a slow growing tumour of one and the same transfer generation. Furthermore, in order to obtain a prolonged outgrowth period, transplantation was also carried out to recipients with a changed immunologic response, so-called hyperimmunized mice. Besides outgrowth period, tumour size and the presence of metacentric chromosomes were recorded. Histologic characterization of the primary tumour and transplanted tumours was also performed.

Material and Methods

Transplantation procedures. Transplantation was always performed to the same sex of the highly inbred strain of CBA mice in order to minimize factors that may favour changes. Therefore only 60±5 days old male mice were used. From a mouse which had received an injection of 14.8 kBq (0.4 μCi) ^{90}Sr/g body weight, a radiation-induced osteosarcoma of predominantly fibroblastic type appeared after 403 days. By serial transplantation in vivo from the primary tumour (NILSSON & ULLBERG 1962) the numerical chromosome progression of 34 succeeding transfer generations was analysed as B series, which had been established previously (Part I). Thus, from the second transfer generation (B2), separate serial transplantation (Fig. 1) was performed from a tumour with an outgrowth period of 21 days (transfer series B) and from a slower growing tumour with an outgrowth period of 44 days (transfer series b). Transplantation was per-

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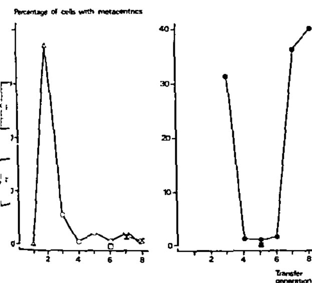
Hyperimm.
72 b5(Hi)
Slow series
44 b3 32 b4 72 b5 39 b6 45 b7 33
B1 17 B2 21 B3 18 B4 19 B5 23 B6 17 B7 14
Fast series
23 B6(H1)
Hyperimm.
17 B7(HiU) 17 B8HU
Back to untreated

Fig 1 Diagram of the transplantations The figures within circles indi ite the age of the tum our used for transplantation

Table 1

H y c nt l h m s me distributi n B = F ist series b = Sl h series b5 (Hi) and B6 (Hi) = Transpl intati n perf rmed t h imi i d e ipient B7 (HiU) = Transp l intatio n perf rmed fro m hyperimmuni ed t untreate l recipients B8 (HiU) C transplantati n t untreated recipients

Transfer genera tion	No of tumours examined	No of cells counted	Chromosome number 39	40	41	43	47	48	49	50	51	52	53	54	55	56	[illegible]	[illegible]
B1	5	125		65 6	1 6												16	[illegible]
B[illegible]	5	125		25 6						0 8						4	7	[illegible]
B3	5	125	2 4	[illegible]									0 8				1	[illegible]
B4	5	125		51 2									0 8	1 6	4 0	5 6	[illegible]	[illegible]
B5	5	125		33 6										0 8	0 9	4 8	[illegible]	
B6	5	125		73 6														
B[illegible]	5	125		39 2														[illegible]
B8	5	125		55													[illegible]	
b3		48		33 3											[illegible] 1	4	[illegible]	4
b4	4	100		[illegible] 0										[illegible] 0	9 0	5 0	[illegible]	[illegible]
b5	5	125		[illegible] 0							0 8		1 6	1 6	15 4	44 0	[illegible]	
b6	5	125		[illegible] 2								0 8		4 8	43	5 6	[illegible]	[illegible]
b7	5	125	1 6	36 0		0 8				0 8		5 6	7 2	36 0	4 0	5 6		
b8	5	125		16 0	0 8	[illegible] 4	0 8	1 6	2 4	4 0	3 2	6 4	13 6	30 4	14 4	1 6	4	
b5 (Hi)	5	125		16 0										0 8	4 0	[illegible]	[illegible]	[illegible]
B6 (Hi)	5	125	0 8	36 8												[illegible]	4	[illegible]
B7 (HiU)	5	125	0 8	[illegible] 0								0 8						
B8 (HiU)	5	125		[illegible] 4												[illegible]	[illegible]	



g ? Percentage of cells with one or more metacentric chromo mes Δ Fast series tran fer generations B1–B? O Fast series ansfer generations B3–B8 □ Transfer generation B6 (Hi) ⧖ ransfer generations B7 (HiU) and B8 (HiU) ● Slow series ansfer generations b3–b8 ■ Transfer generation b5 (Hi)

Table 1 *(cont)*

9	60	61	62	63	64	65	80
3?	?4	4 0	5 6	8 8	4 8	0 8	
3 6	23 ?	10 4	3	1 6	1 6		
6 4	50 4	4 8	1 6	1 6			
1 6	4 8	3 2	5 6	9 6	3 ?	1 6	
0 8	8 0	4 8	20 0	18 4	5 6	1 6	
		0 8	1 0	12 8	0 8		
4	8 0	16 8	1 6	8 8	? 4		
4 0	8 8	1? 8	14 4	4 0			
4 ?	1	16 7	8 3	1			
0							
0 8							
0 8							
	1 6						0 8
1 6	0 8						
1 6	4 8			0 8			
0 8	4	16 0	0 0	1_ 0	3		
0 8	3	10 4	23	3 _	4		
		8 8	13 6	4 0	1 6		

formed from transfer generation b4 also to hyper immunized recipients b5 (Hi) (Fig 1) In the same way transplantation was carried out from transfer generation B5 to hyperimmunized mice B6 (Hi) From this generation two succeeding transplantations were performed but now to untreated recipients B7 (HiU) and B8 (HiU)

Hyperimmunization assays Suspensions from tumours of transfer generation b3 and B4 respectively were separately prepared by pressing the freshly excised tissue through a stainless wire gauze into 2 ml saline solution The suspension was cooled with ice cubes and irradiated with 150 Gy (15 000 R) Doses of 0 2 ml concentrated cell suspension were injected subcutaneously in both flanks of the mice The injections were given three times at weekly intervals One week after the last transfer of heavily irradiated cell material separate transplantation was performed from a tumour of transfer generation b4 to both hyperimmunized (b5 (Hi)) and untreated mice (b5) and also from a tumour of transfer generation B5 to hyperimmunized (B6 (Hi)) and to untreated (B6) (Fig 1) It was not considered necessary to estimate the number of cells transplanted since the main purpose was to analyse the chromosome pattern and not the transplantation antigenicity Extreme care was taken to choose tumour tissue from the same area and of a similar size as when transplanting to untreated hosts

Chromosome analysis The chromosome preparations were obtained according to the air-dry technique described in Part I At the most 5 successful tumours per transfer generation were examined and if possible 25 cells per tumour were analysed altogether 2 148 cells Cells suitable for chromosome counting were selected at magnification 200× and analysed at 1000× Only the percentile chromosome distribution per transfer generation is presented No structural analysis or karyotyping was performed primarily due to limited possibilities with the conventional staining method used

Histologic examination Sections of the tumour were fixed in Stieve s solution embedded and stained according to the van Gieson method or with haematoxylin eosin The definition of tumour classification has been presented previously (NILSSON & RONNBACK)

Results

Registration of chromosome abnormalities The registration of abnormalities was limited to numeri

Table 2

The variation in chromosome distribution often found between the tumours of a transfer generation is illustrated by the chromosome pattern obtained from the tumours of three B generations 25 cells per tumour were analysed

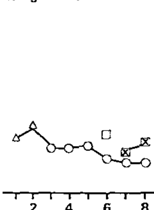
Transfer generation	Tumour No	Growth period (days)	Size (cm)	Chromosome number 39	40	41	53	54	55	56	57	58	59	60	61	62	63	64	65
B1	1 1	16	0 78		19	2						1	1						
	1 [illegible]	17	0 96		14						1			3		3			
	1 3	[illegible]	0 59		19										1		4		1
	1 4	[illegible]	0 91		18											3	1	3	
	1 5	[illegible]	0 72		1[illegible]						1	1	3		2	1	4	1	
B3	3 1	[illegible]	1 40	2	5						1	1	1	12	[illegible]		1		
	3 [illegible]	[illegible]	0 84		1						3	3	3	15					
	3 3	18	1 14		1		1						1	21	1				
	3 4	18	0 88		4							4	3	11	[illegible]		1		
	3 5	[illegible]	0 40	1	17									4	1	[illegible]			
B6	6 1	[illegible]	0 76		25														
	6 [illegible]	1[illegible]	1 [illegible]6		18										1	3	3		
	6 [illegible]	14	1 31		[illegible]											[illegible]	1		
	6 4	14	0 90		15											3	6	1	
	6 5	17	0 90		12											7	6		

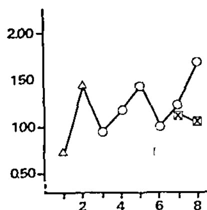
cal chromosome deviations and the occurrence of metacentric chromosomes (Part I) Thus numerous occasionally appearing structural rearrangements were not recorded

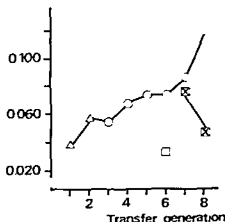
The percentile chromosome distribution per transfer generation is recorded in Table 1 The variation in chromosome numbers between the tumours of a transfer generation is illustrated in Table 2 A diagram of the transplantation appears in Fig 1 The percentage of cells with one or more metacentrics per generation is presented in Fig 2 The mean size and the mean growth period of the tumours per generation are presented in Figs 3 and 4 Finally Figs 5 and 6 show a 58-chromosome cell and a 62-chromosome cell respectively

Chromosome distribution In the first transfer generation (B1) a predominating number of 40 chromosome cells (65 6%) and a wide distribution within the triploid region was observed (Part I) In the second generation (B2) the percentage of 40-chromosome cells had declined to 25 6 per cent while instead an increased number of cells was found within the triploid region From this generation transplantation was carried out both from a fast and from a slow growing tumour By separate serial transplantation the two parallel transfer series (B and b) were established (Fig 1) When comparing the chromosome distribution of transfer generations B3 and b3 generation B3 showed 50 4 per cent [illegible] chromosome cells range 57 to 63 generation [illegible] range of 55 to 63 and flat peaks at 57 and 61 (22 9 [illegible] 16 7% respectively) In contrast to generation [illegible] only 2 1 per cent 60-chromosome cells were f[illegible] The percentage of 40-chromosome cells was [illegible] per cent (B3) and 33 3 per cent (b3) It should b[illegible] ever be noticed that only 48 cells were co[illegible] from the b generation The subsequent B-[illegible] tions (B4–B8) showed a rather high perce[illegible] 40-chromosome cells (33 6–73 6%) With[illegible] loid region the 61 to 63-chromosome cells we[illegible] most frequently appearing types In transfer [illegible] tions b4 to b8 a different chromosomal p[illegible] was observed in the sense that the only [illegible] with a preponderance of 40-chromosome ce[illegible] b4 (59 0%) Also in contrast to the B ser[illegible] b-series was characterized within the triploid r[illegible] by cells with continuously lower chromosome [illegible] bers Peaks were found within the 54 to 57 r[illegible]

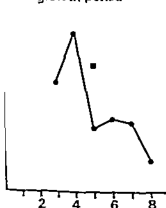
As from transfer generation b4 tran[illegible] was performed also to hyperimmunized [illegible] generation designated b5 (Hi) was obta[illegible] comparing this generation with the corr[illegible] untreated generation b5 rather similar ch[illegible] pattern was found but with the difference th[illegible] b-generation had peaks at 55 and 56 ([illegible] 44 0% respectively) and 24 0 per cent 4[illegible]

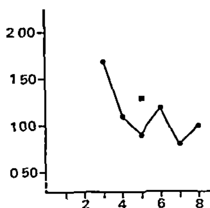


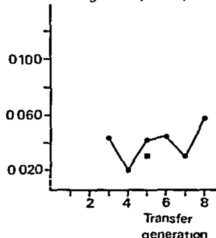




Mean growth period (days) mean size (cm³) and mean h per day (cm) of the B tumours per generation △ Fast transfer generations B1–B³ ○ Fast series transfer generations B3–B8 □ Transfer generation B6 (Hi) ⊠ Transfer generations B7 (HiU) and B8 (HiU)







4 Mean growth period (days) mean size (cm) and mean th per day (cm) of the b-tumours per generation ● Slow s transfer generations b3–b8 ■ Transfer generation b5 (Hi)

e cells while generation b5 (Hi) displayed peaks 6 and 57 (24 8 and 41 6% respectively) and 16 cent 40-chromosome cells Transplantation was performed from generation B5 to hyperim nized recipients (B6 (Hi)) In generation B6 73 6 cent 40-chromosome cells were found in gen ion B6 (Hi) 36 8 per cent only Furthermore in the triploid region generation B6 displayed arrow range of variation (61–64) whereas the ours of the hyperimmunized hosts showed a e distribution (56–64) From generation B6 (Hi) splantation was carried out to untreated hosts s generation denominated B7 (HiU) showed in nparison with the preceding generation B6 (Hi) increased number of 40-chromosome cells (56%)

Within the triploid region a narrower range of variation (59–64) was found The parallel B7 generation on the other hand showed a reduced percentage of 40-chromosome cells (39 2%) Within the triploid region an almost similar range (58–64) as in B7 (Hi) was recorded Finnaly transfer generations B8 and B8 (HiU) had a rather similar chromosome pattern

Mean outgrowth period of the tumours per transfer generation (Figs 3 4) varied to some degree between the series Of interest was a rather distinct variation between the B and the b series and a prolonged outgrowth period when using hyperimmunized mice The B series was characterized by faster growing tumours with an outgrowth period

per generation ranging from 13 8 to 18 6 days while the b series displayed slower growing tumours with a mean value per generation ranging from 16 4 to 54 8 days In generation b5 (Hi) a mean outgrowth period of 44 days was recorded in the parallel generation b5 22 days Also when transplanting from the B series to hyperimmunized hosts B6 (Hi) a prolonged outgrowth period was observed in comparison with the untreated generation B6 The mean value for generation B6 (Hi) was 23 2 days and for generation B6 13 8 days only However when retransplanting from hyperimmunized to untreated hosts once again faster growing tumours appeared The mean outgrowth period of generation B7 (HiU) and the subsequent generation B8 (HiU) agreed well with the parallel generations B7 and B8 The mean values for the B (HiU) generations were 14 8 and 18 0 days respectively and for the B generations 14 0 and 14 2 days

Metacentric chromosomes The percentage of cells with one or more metacentrics per transfer generation is recorded in Fig 2 It should be noticed that besides generation B2 which showed 37 per cent cells with metacentrics high values were also recorded for transfer generations b3 (31 2%) b7 (36 2%) and b8 (40%)

Histologic classification The primary tumour B was classified as an osteosarcoma of predominantly mixed type containing cartilage components of varying degree of differentiation plus tumour bone and in the peripheral regions mainly cells of anaplastic type Samples obtained from tumours of the B and b-generations had continuously more anaplastic cells but with the difference that the b tumours also displayed a focal osteoid formation Because of a failure in preparation no analysis was performed on the tumours of the hyperimmunized generation B6 (Hi) In tumours of generation b5 (Hi) a moderate bone formation was found indicating a higher level of cellular differentiation Along the bone trabeculae the cells resembled osteoblasts and in other areas were still more anaplastic

Conclusions

A primary aim of the present investigation was to test to what extent the selection of tumour used for transplantation may affect the chromosome pattern in succeeding transfer generations but also to analyse whether tumour transplanted to hosts with a changed immunologic response would display a different chromosome pattern in comparison w[...] tumour transplanted to untreated recip[...] Another intention was to examine whether var[...] in outgrowth period could be observed when [...] rate transfer series were established from a slow a[...] a fast growing tumour and when transplanting [...] hyper and nonhyperimmunized recipients T[...] ages of the tumours used for transplantation w[...] except for transfer generation B2 selected so a[...]



Fig 5 A 58-chromosome cell from a tumour of tra[...] tion b5 (Hi)



Fig 6 A 6?-chromosome cell from a tumour of transfer gen[...] tion B5

representative as possible for the generations ler to avoid factors that may interfere with the ation of the results Furthermore the size of eripheral tumour pieces used for transplanta ,as standardized to the utmost possible extent e B and b series displayed a different nosomal progression (Table 1) and primarily n the triploid region The B series was thus cterized by a predominating number of 60 to romosome cells while the b series showed nuously lower chromosome numbers and with s at 54 to 57 Furthermore the tumours of the ies often displayed a higher frequency of 40 nosome cells However even if these results ate differences in chromosomal progression should not be considered as a typical variation slow and fast growing tumour series It should be realized that coincidental changes may have ed this variation especially with regard to often eable differences between tumours of one and ame transfer generation and also between gen ons of a series This circumstance may also ain why no direct effect was observed when splanting tumours to hyperimmunized reci ts even if these tumours for instance displayed aller number of 40-chromosome cells than the ours of the untreated hosts Concerning the n outgrowth period of the tumours per series it found that the mean value for the B series was days and for the b series 29 0 days However e latter series faster growing tumours appeared he late generations When transplanting to erimmunized recipients a prolonged outgrowth od was observed in comparison with the un ted parallel generations but it was also noticed when retransplanting to untreated recipients er growing tumours were obtained Thus it can be ruled out that there may be a certain variation sed by difference in the immunologic response that this variation is not quite uniform as larities were found between untreated and some erimmunized recipients Nevertheless the pres results should indicate the need for some cau when evaluating at least transplanted radiation uced tumours

SUMMARY

Highly inbred CBA mice were used The registration of chromosome abnormalities was limited to numerical de viations and the occurrence of metacentric chromosomes By separate serial transplantation from a ^{90}Sr induced os teosarcoma two parallel transfer series (B and b) were established From these series transplantation was also performed to hyperimmunized hosts B (Hi) and b (Hi) Besides differences in mean outgrowth period between B and b generations a variation in chromosome pattern was observed However this variation should not be evaluated as a typical chromosomal progression of fast and slow growing tumour series

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GRANULOSA AND THECA CELL TUMORS

Incidence and occurrence of second primary tumors

ELISABET BJÖRKHOLM and CLAES SILFVERSWARD

Granulosa and theca-cell tumors constitute the most common types of the ovarian gonadal stromal tumors The malignant potential of granulosa-cell tumors is a matter of debate and figures given in the literature for recurrences or metastases vary from less than 10 to more than 33 per cent (MORRIS & SCULLY 1958) They contain granulosa and theca cells in varying proportions (TEILUM 1971) Pure theca-cell tumors thecomas are generally benign (NORRIS & CHORLTON 1974) The ratio of granulosa to theca cell tumors varies in different series BIDDLE (1952) in a large compilation found the ratio to be 4 1 others have reported 1 1 The incidence rates for granulosa cell tumors in different countries and races vary from 0 05 to 1 70 per 100000 female population (KOLSTAD & BEECHAM 1975) This large range is due at least in some measure to differences in the reporting of these lesions for example some reports seem to include pure theca cell tumors others not Incidence rates for thecomas are difficult to ascertain The occurrence of endometrial carcinoma in patients with feminizing tumors of which granulosa and theca cell tumors constitute the main part has been well documented INGRAM & NOVAK 1951 MANSELL & HERTIG 1955 GREENE 1957 GUSBERG & KARDON 1971)

The incidence of granulosa and theca-cell tumors in the total Swedish female population was analysed and the numbers of new primary malignant tumors reported in women with granulosa and theca-cell tumors compared with the expected numbers by using data from the Swedish Cancer Registry The results are now reported

Material and Methods

Since 1958 Sweden has operated a nation wide Cancer Registry to which it is compulsory to report all malignant neoplasms and also tumors of certain types irrespective of whether they are classed as benign or malignant Granulosa and theca-cell tumors belong to the last mentioned category (Cancer Incidence in Sweden 1959–1965)

In the Registry granulosa and theca-cell tumors and certain other types of ovarian neoplasms (mostly virilizing tumors and Brenner tumors) are assigned the same microscopic code A list of women with this special code was obtained The original microscopic reports (furnished by the different hospitals) on which the diagnoses were based are filed in the Registry After analysing these files it was found that 687 cases should be classified as granulosa-cell tumors 249 as theca-cell tumors and 72 cases belonged to neither of these categories The series thus comprised 936 cases of granulosa and theca-cell tumors reported to the Swedish Cancer Registry between 1958 and 1972 of these 4 per cent were detected at autopsy The list from the Cancer Registry provided information on the date of birth the name of the patient the date of diagnosis the mode of diagnosis (operation or autopsy) the reporting hospital and the date of diagnosis of any other malignant disease (until December 1973) In the case of death the date and cause of death were

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given (until December 1976) A closing date for collecting the series was set to December 1973

From the Registry list—now containing only granulosa and theca cell tumors—every tenth case was taken for a morphologic re-evaluation The hospitals reporting the patients were asked for the histologic specimens (slides) on which their initial diagnoses were based This material was received for 87 cases of the 93 requested These 87 cases will be referred to as the 10 per cent sample If carcinoma of the endometrium had also been reported in a patient the endometrial biopsies were sent for as well As the discrimination between granulosa and theca cell tumors in the Cancer Registry was objectionable all the calculations except the estimations of incidence rates for the two tumor types were based on the total series that is granulosa and theca cell tumors taken together

Statistical methods The average annual crude (all ages) incidence rate was computed from the average annual number of cases 1958 to 1972 and the mean female population of 1965 was used as the mean population for the period

The age specific person years at risk for contracting a new malignant disease was calculated from date of diagnosis of the ovarian tumor to date of death or the closing date of the series Age specific person years at risk for developing an endometrial carcinoma was computed in the same way with the exception that for patients developing endometrial carcinoma the date of diagnosis of that disease was stop date By applying the age specific incidence rates obtained from Cancer Incidence in Sweden 1965 (the median year of the series) for different malignant diseases to the number of person years at risk the expected numbers of these tumor types were calculated (Cancer Incidence in Sweden 1965) The life time risk of developing an endometrial carcinoma was estimated by using information from life tables of Sweden for 1963 to 1967 and from Cancer Incidence in Sweden 1965

The ratio of observed to expected numbers of malignant lesions was defined as the relative risk 95 per cent confidence limits for the relative risk were given based on the Poisson distribution

Age specific incidence rate was calculated for each 5 year group as the annual number of cases per 100000 of the mean female population in that age group An age standardized incidence rate was calculated for each year The figures were adjusted for age to a standard population (the 1970 Census)

Table 1

Cases initially diagnosed as granulosa cell tumors and at review considered as other tumor types

Diagnosis on review	No of cases
Arrhenoblastoma	[illegible]
Serous cystadenocarcinoma	[illegible]
Insulinoma	1
Carcinoid	1
Mesothelioma	1

The time trend was calculated over the per from 1959 onwards (omitting 1958 the first year the Cancer Registry operated) using sliding mean the logarithms of the age standardized incidence each year

Results

The 87 reviewed cases i e the 10 per sample agreed well with the whole Cancer Reg series concerning mean ages at diagnosis 57 an years respectively and the ratio granulosa to th cell tumors based on the initial reports 3 0 1 2 8 1 respectively On the basis of the review per cent of the 87 cases belonged to either the gr losa or the theca-cell tumor group At the re only 59 per cent of the cases initially reporte granulosa-cell tumor were considered to belon that group 29 per cent were re-classified as th cell tumors and 11 per cent were considered t other types of tumors (Table 1) In one case dia sis was not possible because of extensive necro Ninety five per cent of cases with a reported gnosis of theca cell tumor were after re evalua of the histologic specimens considered to belon that group Five per cent were re-classified as gr losa-cell tumors as they contained a certain am of epithelial strands of the granulosa-cell tu type Applying these figures to the total serie 936 patients 421 should be considered as granul cell tumors and 431 as theca cell tumors Based these last mentioned figures the averag an crude incidence rate 1958 to 1972 of granulosa tumours was 0 72 and of theca-cell tumors 0 74 100000 female population the 95 per cent fidence limits in both instances are 0 55 and 0 90

The mean age at diagnosis for the total se was 58 years and the range 7 months to 89 y (Fig 1) The age specific incidence rate for

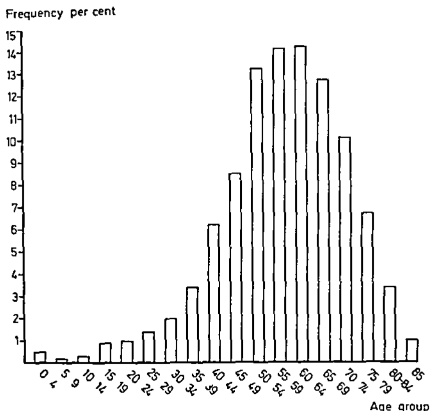


Fig 1 Age distribution of 936 women with granulosa and theca cell tumors reported to the Swedish Cancer Registry 1958 to 1972

roup is given in Fig 2 An almost linear increase etween 35–39 years and 65–69 years is found The ge standardized annual incidence rate and the end appear in Fig 3 There seems to be an increased incidence until 1966 after which time it mained stationary The correlation with time is ositive (r=0 4) but not significant p=0 25

After the diagnosis of the granulosa or theca cell umor the 936 women developed 55 new malignant iseases excluding other ovarian tumors (Table 2) e expected number is 42 8 (no statistically significant difference) In addition as many as 62 malignant tumors were found at the same time as the varian tumor the majority (45) being endometrial arcinomas The mean age at diagnosis of the varian tumor for the 45 women with concomitant ndometrial carcinoma was 67 years Five women ith endometrial carcinomas reported after the iagnosis of the ovarian tumor had a mean age at iagnosis of the ovarian tumor of 43 years The ifference in mean age at diagnosis of the ovarian umor for the two endometrial tumor groups is statistically significant (p<0 001) The mean interval etween the ovarian diagnosis and the diagnosis of the endometrial carcinoma was 8 9 years (range 4 6–13 5 years) The expected number of cases of endometrial carcinoma based on person years at risk was 2 6 In addition 8 women had a report of an endometrial carcinoma before the diagnosis of the ovarian tumor If the life time risk of developing an endometrial carcinoma is calculated for the patients with granulosa or theca cell tumors (according to the microscopic review) the expected number would be 13 3 The total number of observed cases was 58 detected before concomitant with and after the diagnosis of the ovarian tumor This gives a relative risk of 4 4 95 per cent confidence limits (3 3–5 6)

Nine women with an endometrial carcinoma reported to the Cancer Registry were found in the 10 per cent sample In 6 cases it was possible to confirm the diagnosis at review of the endometrial biopsies and one case was considered not to have an endometrial carcinoma Two cases belonged to that group which was not considered to have this kind of ovarian tumor at the re evaluation of the histologic specimens

Fifteen mammary carcinomas were found after

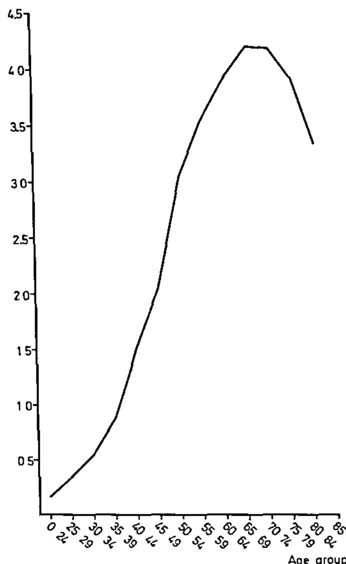


Fig 2 Age specific incidence rates for granulosa and theca-cell tumours 1958 to 197ˀ per 100 000 of the mean female population

Table 2

New malignant tumors reported concurrently with after diagnosis of ovarian neoplasm and the expect numbers

Tumor site	Observed number		Expec (based years at risk time of diagn of ovarian tumor)
	Reported after the ovarian tumor	Reported with the ovarian tumor	
Stomach	2	[illegible]	3 6
Small intestine	1	0	0 7
Colon	6	4	4 1
Rectum	3	1	1 9
Biliary ducts and liver	[illegible]	0	1 6
Pancreas	3	0	1 6
Breast	15	4	10 3
Uterine body	5	45	[illegible]6
Uterine cervix	0	3	2.4
Kidney	1	0	1.5
Lower urinary tract	1	0	1 0
Melanoma of the skin	1	0	0 6
Skin	1	0	0 8
Brain	2	0	1 3
Thyroid gland	1	-	0 5
Other and unspecified locations	3	1	1 4
Malignant lymphoma	5	0	1 0
Multiple myeloma	1	0	0 6
Leukemia	2	0	1 0
All sites except ovary	55	62	4[illegible] 8
All sites except ovary and uterine body	50	17	40 [illegible]

the diagnosis of the ovarian tumor compared with 10 3 expected This difference is not statistically significant However if concomitant breast carcinoma is included the difference is significant the relative risk is 1 8 95 per cent confidence limits (1 1–2 9)

The difference between observed and expected number of cases of carcinoma of the colon after diagnosis of the ovarian tumor was not statistically significant but if concomitant colon carcinomas were included the difference is statistically significant the relative risk being 2 4 95 per cent confidence limits (1 2–4 4)

The same conditions apply to the thyroid carcinomas where no statistically significant difference between observed and expected cases was when concomitant cases were not taken in count However if tumors concomitant w ovarian tumor were included the difference is tically significant the relative risk being 6 0 cent confidence limits (1 2–17 5)

The relative risk of contracting a ma lymphoma was 5 0 95 per cent confidence (1 6–11 7) thus the difference between ob and expected cases is statistically significa concomitant malignant lymphomas wer served

No statistically significant difference b observed and expected numbers of leukem found

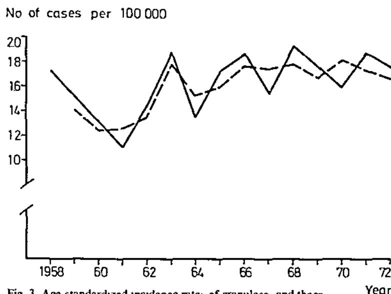


Fig 3 Age standardized incidence rates of granulosa and theca cell tumors 1958 to 1972 per 100 000 of the mean female popula tion (solid line) and the trend (broken line)

Discussion

The calculations have been based on Registry ures The reliability of such data may be dis ssed and as the Registry has many contributors e Swedish Cancer Registry is likely to contain me erroneously diagnosed cases (Cancer Inci nce in Sweden 1973) The extent of possible rors in the diagnoses has not been analysed ex pt in special research projects based on small imbers of selected types of tumors (MALEÇ et ll 1977) No information on treatment was given spite of these elements of uncertainty it is prob ble that analyses of the present kind may shed ht on the etiology of malignant tumors

The review of the 10 per cent sample disclosed at only few errors of diagnosis were made when anulosa and theca-cell tumors were allotted one and the same group However the differ ntiation between these two tumor types was less liable Most errors affected the granulosa-cell mor diagnosis Based on the figures after reclassi cation the incidence rates of granulosa and theca ell tumors were found to be almost equal In par cular KOLSTAD & BEECHAM mentioned the re arkably high incidence of granulosa-cell tumors in weden as compared with other countries This may some extent be explained by the fact that in weden thecomas have been included in the calcula ons and to a minor part by errors made in the istologic classification After making corrections for the number of thecomas and incorrect diagnoses the incidence rate of granulosa-cell tumors in Sweden was still high compared with most other countries and approximately the same as that in Norway (KOLSTAD & BEECHAM) No change in incidence rates of granulosa and theca-cell tumors combined was found from 1966 onwards The possible increase from 1959 to 1965 may depend on some uncertainty in the reporting of these tumors during the first years that the Cancer Registry operated It is difficult to estimate the true incidence of these relatively benign neoplasms possibly at least some cases are not diagnosed This may espe cially be true for the benign thecomas Very few tumors in the present series were first diagnosed at autopsy This fact may simply reflect a low fre quency of post mortem examinations The number of endometrial carcinomas in the 10 per cent sample was too small to draw any statistical inferences

The mean age at diagnosis for this series is similar to that given previously (BURSLEM et coll 1954 DIDDLE) It has been suggested that the maximum incidence of these feminizing ovarian tumors about the menopause should support a theory that pitu itary gonadotrophins stimulate latent components of the ovary to tumor production (BURSLEM et coll) In fact increased incidence rates were found with advancing age especially so from menopause at least when the very old age groups were not taken into account This supports the theory mentioned

A nation wide series of granulosa and theca-cell tumors with a known back ground population and access to reports of new primary tumors made this analysis possible A comparison with the expected number of the relevant tumors based on the age specific incidence rates for the Swedish female population has been possible An analysis of new primary tumors may however be biased for a variety of reasons One is the possibility of detecting one tumor as a consequence of the treatment of another If as in the present material the ovarian tumor is comparatively benign and sometimes has a long duration before being diagnosed (BJÖRKHOLM & PETTERSSON 1980) the evaluation of concomitant tumors must be handled with special care It is difficult to calculate the actual person years at risk and subsequently the expected number of tumors Most patients having had one malignant disease will also be under careful medical supervision and if a new neoplastic lesion appears the possibility of detection must be greater than usual

Granulosa and theca-cell tumors appear to have the capacity of producing endometrial change through prolonged unopposed or unmodified hormone stimulation by estrogen alterations or other endocrinopathies the progress from normal endometrium to carcinoma under the influence of continuous endogenous or exogenous estrogen stimulation has been described (GUSBERG & KARDON) The women in the present series ran a four times increased risk of developing an endometrial carcinoma as compared with the life time risk for the general population This method of calculating gives a considerable under estimation of the true relative risk However the life time risk was chosen for the comparison because of the complex problem of handling the large number of concomitant tumors and thus of reliably computing the expected numbers On the other hand as most of the endometrial carcinomas were concomitant with the ovarian neoplasm treatment could not have had any major influence The number of endometrial carcinomas observed after the diagnosis of the ovarian tumor seems to be high considering that an unknown but certainly not negligible number of women must have been hysterectomized at time of diagnosis of the ovarian tumor Those who developed endometrial carcinoma after the ovarian diagnosis were significantly younger at time of diagnosis of their ovarian tumor than were the women with concomitant endometrial carcinoma Here the usual concept that continuous estrogen stimulation changes the en metrium into pre-cancerous and cancerous les does not hold true The probable excess estr production was discontinued by the removal of ovarian tumor and some 9 years later an endome carcinoma developed While it is not known whe a recurrence of the ovarian tumor occurred in t women no deaths appeared among them from cause These patients may also have received r tion therapy There may be a common etiol factor perhaps genetical or hormonal that ma fluence the development of both the ovarian an endometrial tumor

An increased risk of new primary tumors has found in patients with carcinoma of the lip (HORN & JAKOBSSON 1964) and ovarian carcin (REIMER et coll 1978) as first primary tur REIMER et coll reported an increased risk of se primary carcinomas of the uterine corpus c bladder breast and hematopoietic system in fo up surveys of patients with ovarian carcinom types In the present series carcinoma of the br colon and thyroid all occurred in excess or tumors concomitant with the ovarian neoplasm taken into account The women were found to a significantly higher risk of developing mali lymphomas but no over risk for leukemias wh in concordance with the mentioned repor ovarian carcinoma

SUMMARY

An analysis of 936 women with granulosa and t cell tumors reported to the Swedish Cancer Re between 1958 and 1972 showed crude annual inci rates for these lesions of 0 72 and 0 74 respectivel 100 000 of the female population unchanged durin period The age specific incidence rates both g taken together increased almost linearly from 35 years The women displayed an increased risk d veloping endometrial carcinoma and malignant lymp possibly also breast colon and thyroid carcinoma

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PROLACTIN SECRETING PITUITARY ADENOMA

Observations in irradiated patients

A DE SCHRYVER D VANDEKERCKHOVE and G DEBRUYNE

Although isolated descriptions of galactorrhea and menorrhea (or impotence) associated with the exis nce of a pituitary tumor were to be found in the erature more than half a century ago (BROWN 1925 MENEI 1928 KRESTIN 1932) FORBES et coll 954) could define a syndrome characterized by alactorrhea amenorrhea and low urinary gonado ophines in 15 non acromegalic patients (8 of these ith evidence of a pituitary tumor) It was sub quently known as the Forbes Albright syndrome he tumors associated with this clinical entity were first thought to be chromophobe (FORBES et coll) it subsequently when more refined cytochemical ethods were applied were very often found to ontain erythrosinophilic cells (HERLANT et coll 965 PEAKE et coll 1969 LANDOLT 1973) There is ow good evidence that (as had already been uggested by FORBES et coll) the symptoms in these atients are to be explained on the basis of the ncreased prolactin secretion by the gland or the denoma when present (FORSYTH et coll 1971 HORNER et coll 1974) In fact the incidence of denomas in these patients is probably higher than would be expected on the basis of conventional ateral skull films VEZINA & SUTTON (1974) found adiologic evidence of a pituitary tumor in 20 patients with serum prolactin increase 14 having a ella of normal size All tumors were confirmed at urgery

The main reason why these patients seek medical advice is usually sterility (in men impotence) for which the efficacy of 2 bromo-α-ergocryptine (bromocriptine Parlodel) medication has been well documented (THORNER et coll SHEARMAN & FRASER 1977) However some cases do not respond satisfactorily to medical treatment (THORNER et coll) and more important a risk of accelerated tumor expansion exists during pregnancy (GEMZELL 1975 SHEARMAN & FRASER DE GENNES et coll 1978) with potentially grave ophthalmic complications as a consequence

The short term (1 5 to 3 years) results in 6 patients treated with external irradiation are now reported and some of the problems relating to these data are discussed

Material and Methods

The 6 women were referred by the Department of Gynecology of this hospital for a sella enlargement high prolactin levels reaching 300 ng/ml or more ophthalmologic symptoms or primary resistance to bromocriptine medication or any combination of these Some of their relevant clinical data are summarized in Table 1 All had been treated previously with various drugs including clomiphene human menopausal gonadotrophine human chorionic gonadotrophine and bromocriptine for periods varying from 2 to 7 years In Case 3 the symptoms developed after discontinuation of oral contracep-

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Table 1

Pre irradiation data

Case No	Sex	Age at irradiation	Amenorrhea (years)	Galactorrhea	Sella abnormality	Visual fields	Previous treatment period (years) with ovulation induction or bromocriptine
1	F	39	2	–	Erosion of floor	Bitemporal sup quadrianopsia	7
2	F	[illegible]	4	+	Erosion of floor	Normal	4
3	F	[illegible]	[illegible]	+	Enlarged	Normal	[illegible]
4	F	26	[illegible]	+	Erosion of floor	Normal	2
5	F	27	4	+	Erosion of floor	Normal	[illegible]
6	F	6	6	+	Enlarged	Incipient sup quadrianopsia	[illegible]

Table 2

Mean plasma LH FSH TSH GH and cortisol levels during radiation therapy

	Normal range	Day 0 (pre irr)	Day 4	Day 8	Day 15	Day 22	Day 29	Day 36	Day 43
LH	4–20 mIU/ml	9 6	10 5	8 3	6 1	6 5	7 5	7 0	3 8
FSH	6–15 mIU/ml	12 7	17 1	16 1	10 9	7 7	9 7	11 4	8.3
TSH	<5 μU/ml	9 4	–	3 5	3 9	2 1	2 6	2 8	5.5
GH	1–5 ng/ml	0 1	–	0 25	0 3	2 8	0 7	1 8	0 1
Cortisol	80–280 ng/ml	251	–	139	150	129	210	153	115

tive treatment All had an abnormal sella strongly suggestive of an intrasellar adenoma uni or bilateral erosion of the sella floor to unambiguous enlargement of the sella During this same period (Jan 1 1976 to Sept 30 1978) a further 23 women with hyperprolactinemia and amenorrhea or short luteal phase (14 among them with galactorrhea) were successfully treated medically and irradiation did not have to be considered In only 2 of these was a sella floor asymmetry found

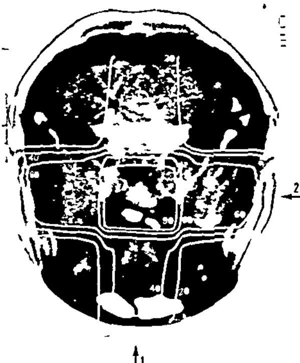
Hormone assays Prolactin was assayed by means of a double antibody radio immunoassay technique using the NIH reference preparation as a standard Serum prolactin levels of 5 to 25 ng/ml were considered normal Other pituitary hormones were assayed in 3 patients (Cases 1 2 5) and included gonadotrophines (FSH) luteinizing (LH) thyroid stimulating (TSH) and growth (GH) hormones and cortisol Their normal (fasting) ranges are given in Table 2 For FSH and LH the figures relate to the follicular phase of normally menstruating women

Radiation therapy Patients were treated with 8 MV photons from a Philips SL75 linear accelerator using a 3 field arrangement (Fig 1) Portal measured 4 cm × 4 cm and their position center on the pituitary fossa was carefully checked by means of routine simulator films Daily pituitary doses of 1 8 Gy were administered up to a total of approximately 50 Gy in 5½ weeks (cf Table 3 for details) All 3 fields were treated each day

Patients were controlled at frequent regular intervals but at least once every month and hormone assays were repeated as a rule to assess the effe of subsequent medication which was continued all patients

Results

Clinical data In 3 of the 6 patients irradiated (Cases 1 3 5) it would seem as if radiation thera followed by maintenance bromocriptine treatment had contributed to the reduction of serum prolactin to normal levels with the result that 2 of them eve tually became pregnant 6 and 9 months respec tively after completion of irradiation Each of these two gave birth to a normal child in the third



1 Example of radiation dose distribution

mal menstrual cycle was re-established (with gnancy no longer wanted) All 3 are still on main ance bromocriptine medication (5 to 7 5 mg ly) In Case 1 the bitemporal superior quad nopsia disappeared The remaining 3 patients do seem to have benefitted from irradiation In se 6 the adenoma continued to grow with ag vation of her visual symptoms until one year er the irradiation course surgery had to be per formed The lesion (a chromophobe adenoma) was removed trans sphenoidally during an uneventful operation She is still under 5 mg bromocriptine and both her vision and prolactin levels have normalized as well as her menstrual cycle (2 years after surgery) The other two (Cases 2 4) are still amenorrheic one and 3½ years respectively after irradiation but no further treatment other than bromocriptine *is being considered at the moment*

Radiation induced hormone level changes Before irradiation serum prolactin levels varied widely in the 6 cases and ranged from 70 to 790 ng/ml (median 255 ng/ml Fig 2) in contrast to the 23 patients who responded primarily to medication and were not ir radiated (median 57 ng/ml) The 3 responders do not seem to have had any particularity in common 2 of them had high initial prolactin levels (Cases 1 and 3) while Case 5 only had moderately elevated prolac tinemia Their response to the radiation therapy (Figs 2 3) was comparable to that of the others Among the non responders only in Case 2 could it be said that a rather poor effect on prolactin levels was obtained (but no worse than that for Case 5 who responded) On the other hand in Case 6 the prolactin levels were more than halved during the radiation course although without clinical result On the evidence of this small group of patients it would seem that prolactin variations during radiation therapy are probably of little help in pre dicting the result of the treatment During ir radiation prolactin levels showed after an initial rise in some a downward trend in every patient with end levels of 81 68 67 43 and 31 per cent of

Table 3

Treatment data and clinical results

Case No	Irradiation data	Post irradiation bromocriptine maintenance treatment (mg)	Clinical result	Final prolactin levels (ng/ml)
1	55 Gy/42 d	7 5	Normalization of cycle disappearance of quadrianopsia	15 2
2	50 Gy/37 d	12 5	No response	52 0
3	50 Gy/44 d	7 5	Pregnancy	12 1
4	50 Gy/42 d	7 5	No response	98 1
5	45 Gy/37 d	5 0	Pregnancy	23 4
6	50 Gy/42 d	5 0	Progressive growth of adenoma with deterioration of vision	<2 (post surgery)

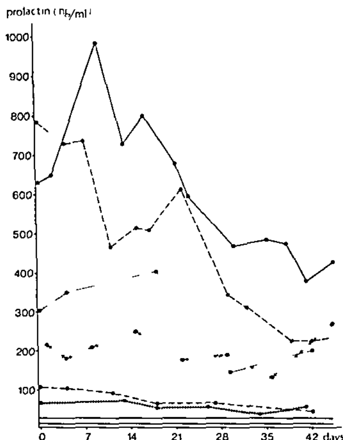


Fig 2 Serum prolactin variations in absolute figures during irradiation (45 Gy/37 days to 55 Gy/4? days) ▭ normal value Case 1 (---) Case 2 (+++) Case 3 (—) Case 4 ( ) Case 5 ( ) Case 6 (- -)

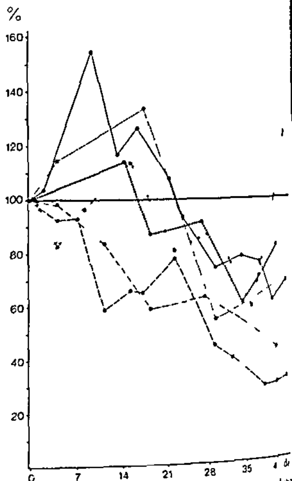


Fig 3 Serum prolactin variations (in per cent of pre irradiation value) during irradiation Symbols as in Fig 2

pretreatment values Case 2 ended up with a higher than pretreatment level (125%) and proved a non responder (even to bromocriptine) Among the others no correlation was found between the degree of serum prolactin decrease and clinical response

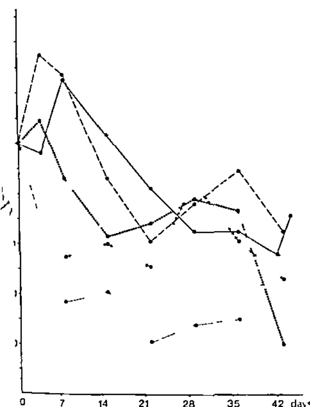
In 3 patients other pituitary hormones were as sessed during treatment for comparison with prolac tin values (Table 2 Fig 4) It would seem that (with the exception of GH which varied widely) FSH LH TSH and cortisol also had a similar downward trend as did prolactin although by the end of the treatment they were still by and large well within normal range

Discussion

Radiation therapy as a primary treatment for pro lactin secreting pituitary adenoma has been used by KLEINBERG et coll (1977) Their results did not seem promising Of 8 patients treated none became pregnant none of 6 amenorrheic patients had a re sumption of their menses although galactorrhea dis appeared in 3 But prolactin levels were reduced [to] an average of 26 per cent of pretreatment value[s] The authors concluded that they could find no clea[r] superiority of either trans sphenoidal hypophys[ec]tomy or irradiation as a mode of treatment in the[se] patients SHEARMAN & FRASER treated 5 patien[ts] with (intrasellar) microadenomas with external [ir]radiation but found it too early to determine th[e] result of this treatment

The present data admittedly on only 6 patient[s] show that prolactin secreting adenoma can resp[ond] to irradiation probably in a substantial proportion [of] cases They do not obviously permit any apprai[sal] on the definitive value of radiation therapy in t[he] management of this condition much less its com[parison] with primary surgery They also raise a fe[w] further questions which it may be of interest [to] comment upon in brief

The first question is whether the radiation-[in]duced prolactin decrease is due to a direct effect [on] the adenoma or to an indirect hypothalam[ic]



g 4 Variations in serum FSH (---) LH ( ) TSH ( ) ortisol (+++) mean of 3 patients and serum prolactin (—) an of 6 patients during radiation therapy in per cent of pre ir diation value

ediated effect It has been suggested at least for ome cases of pituitary tumor that the mechanism ggering the prolactin hypersecretion might be re ted to interference with the pituitary stalk or ypothalamus (FRANTZ et coll 1972 FRANKS et oll 1977) and to the resulting loss of its prolactin hibitory action If for the sake of argument it is sumed that such a mechanism were at all involved its pathogenesis then it is of course conceivable that this particular mechanism might be affected by idiation therapy The data presented here do not ermit a definite conclusion one way or the other However in view of the relatively early repercus on of the irradiation course on serum prolactin etectable at dose levels not considered particularly igh (20 to 30 Gy) it may well be asked whether this nay not have been mediated through changes in uced at the hypothalamic rather than at the denoma level Growth hormone levels in ac omegalic patients can respond to irradiation but this eens to be a process that will take months to years ROTH et coll 1970) Pituitary irradiation for Cush ngs disease may induce a decrease of serum ACTH but even this process will take several months (ROSS et coll 1979) LARKINS & MARTIN (1973) described a case of hypopituitarism after radiation therapy developing in a patient given 65 Gy for a carcinoma of the maxilla and in whom they felt the pituitary insufficiency might have been of hypothalamic origin However and although very little is known about the sensitivity of the hypothalamic structures (most of which are prob ably comprised within the target volume whenever the pituitary is irradiated) it seems unlikely that a dose of 50 Gy could result in a functional boost i e an increase in prolactin secretion inhibition The observed serum prolactin decrease seems most likely to be explained on the basis of a direct effect of the radiation on the pituitary cellular level

The second question is whether the radiation in duced decrease of prolactin levels in these patients is a specific phenomenon or whether the other pituitary hormones are also influenced as well as the normal glandular function The integrity of the var ious other pituitary axes could be confirmed only through the use of special stimulation tests which were not performed However from a purely clinical point of view the answer to these questions was simple at least in a short term perspective no symptoms suggestive of impairment of any of the other pituitary functions were ever noticed The sensitivity of normal adult pituitary tissue and hor mone producing mechanisms have been known for a long time to be quite low A recent literature review (FUKS et coll 1976) was found to mention only 2 adult cases of radiation induced hypopituitarism which was diagnosed 7 and 8 years after irradiation (65 and 60 Gy respectively) The authors suggested the possibility of long term damage on the basis of cumulative post mitotic cell death in a slow renewal type of tissue Apart from the extreme rarity of reports of clinically established cases an analysis of 29 patients surviving as adults for 10 to 30 years after radiation therapy for malignant nasopharyngeal tumours and in whom the dose to the pituitary was estimated as ranging from 17 to 68 Gy did not reveal any case of unequivocally impaired pituitary function at least with the tests then in use (DE SCHRYVER et coll 1973) Therefore the risk even long term for clinically relevant injury to the glan dular tissue within the irradiated normal pituitary probably is quite small

It must be noted that the serum levels of most other pituitary hormones tested (the exception being

GH) decreased Since roughly similar curves were found for FSH LH TSH and cortisol this can hardly be fortuitous and irradiation must therefore in all probability and to some extent interfere with the secretion of these various pituitary hormonal products as well However either these decreased levels are merely transient or if not the decrease is not sufficient to affect their peripheral targets

The third question is whether the radiation induced prolactin decrease is a dose related phenomenon and the proportion of responders increased by increased dosage Again the limited number of cases does not allow a definite answer The patient who received the lowest dose (45 Gy in 37 days) responded as did the one with the highest dose (55 Gy in 47 days) Whether a higher dose than 50 Gy in 42 days would have cured the 3 remaining patients is moreover somewhat irrelevant since the choice of an optimum dose is wholly dominated by the sensitivity of the adjacent neural structures in general and by that of the optic pathways in particular The optimum dose in fact is the highest dose which will not induce injury to the optic nerve For many years recommended dose levels have usually ranged between 45 and 55 Gy in 4 5 to 5 5 weeks As more and more experience is accumulating it has become increasingly clear that a total target dose not surpassing 50 Gy in 28 daily fractions is unlikely ever to exceed nervous tissue tolerance and to induce neurologic (mainly ophthalmic) complications (ARISTIZABAL et coll 1977) In order to keep radiation therapy of pituitary adenoma a safe procedure (which seems mandatory in view of the benign character of most of these lesions) due consideration must be given to the proper time dose parameters and allowance made for a number of treatment failures the true proportion of which has yet to be determined

SUMMARY

Six women with clinical biochemical and radiologic evidence of a prolactin secreting pituitary adenoma were irradiated with 50 Gy in 5½ weeks In 3 of the 6 patients irradiation preceding maintenance bromocriptine medication was followed by a normalization of serum prolactin levels which resulted in a normal pregnancy in 2 and reestablishment of the menstrual cycle in the third In 5 of 6 patients serum prolactin was substantially reduced by the end of the treatment but without reaching normal levels although no correlation was found between the degree of this decrease and the clinical result

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PRIMARY MUCOSAL MALIGNANT MELANOMA

Appraısal of role of radıatıon therapy

YUNG H SON

Pnmary malıgnant melanoma rarely arıses ın e oropharyngeal gastroıntestınal or urogenıtal ucosa and even more rarely ın the central nervous stem The prognosıs of malıgnant melanoma of e skın ıs largely ınfluenced by the hıstogenetıc fea res thıckness sıze of the lesıon degree of lympho tıc ınfiltratıon and lastly type of surgery or adju ınt ımmunochemotherapy (GOLDSMITH et coll ı70 SHAH & GOLDSMITH 1970 BLUMING et coll ı72 RIPPEY 1973 HANSEN & MCCARTEN 1974 IORTON et coll 1974 COHEN et coll 1977 HOLMES coll 1977 EL DOMEIRI et coll 1978)

Meanwhile malıgnant melanoma of the mu ɔus membrane ıs generally regarded hıghly le ıal ırrespectıve of therapeutıc modalıty due to › hıtherto controversıal hıstogenesıs potentıal ulucentrıcıty as well as caprıcıous metastasızıng ıpabılıty and hıgh local recurrence rate (STOUT LATTES 1957 DE LA PAVA et coll 1963 RAVEN DAWSON 1964 DESAI & CAVAUGH 1966 TEARNS et coll 1966 COLLANTES et coll 1967 ACK & OROPEZA 1967 PICCONE et coll 1970 AUFE & BERNSTEIN 1971 DAW 1972 FRABLE t coll 1972 DEUTSCH et coll 1974) Although ıt ıs ır from convıncıng the therapeutıc norm tends to ıor radıcal surgery throughout the lıterature MINO et coll 1948 DAS GUPTA & D URSO 1964 ɔESAI & CAVAUGH NORRIS & TAYLOR 1966 TEARNS et coll COLLANTES et coll PACK & ɔROPEZA YACKEL et coll 1970 BINGHAM et coll 971 LAUFE & BERNSTEIN MORROW & RUTLEDGE 97? DEUTSCH et coll MUSHER & LINDNER 1974)

Incıdence of prımary malıgnant melanoma of the female genıtalıa ıs approxımately 0 1 per 100 000 female populatıon 3 per 1 000 skın melanomas or less than 3 per cent of all malıgnancıes of the vagına (DAS GUPTA & D URSO DEUTSCH et coll RAGNI & TOBON 1974) To date about 100 cases of vagınal malıgnant melanoma have appeared ın Englısh lıterature (MINO et coll DAS GUPTA & D URSO NORRIS & TAYLOR LAUFE & BERNSTEIN DAW DEUTSCH et coll)

DAS GUPTA & D URSO reported 3 cases to the exıstıng 30 cases and observed a 5 year survıval of 9 per cent (or 3 of 33) They advocated complete vagınectomy vulvectomy and groın dıssectıon for a mıd thırd lateral vagınal lesıon Less emphatıcally dıd they advocate pelvıc exenteratıon for a superıor thırd vagınal lesıon

MULLANEY (1961) acceptıng only 15 of the 24 reported cases ın the lıterature added one case of her own After revıewıng 13 cases NORRIS & TAYLOR reported an 8 per cent 5 year survıval by sımple to wıde excısıon wıth pelvıc node dıssectıon COLLANTES et coll after addıng 3 new cases concluded that local excısıon ınvarıably faıled both at prımary and dıstant sıtes ın a short tıme DESAI & CAVAUGH were dubıous about extended radıcal surgery because of unpredıctabılıty ın the behavıor of the dısease

DEUTSCH et coll collected 74 cases from the lıterature and added 5 cases of theır own Conced

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ing to a high incidence of local and pelvic recurrence after radical surgery they mildly advocated radical surgery for a better chance of local control over simple excision alone The 3 cases subjected to radical surgery died in 1½ to 4 years while the 2 cases treated by local excision and radiation died within 9 months

According to FENN & ABELL (1973) and RAGNI & TOBON various surgical modalities ranging from simple excision to radical surgery equally gave rise to a 9 per cent 5 year survival Reiterating therapeutic skepticism toward radical surgery LAUFE & BERNSTEIN observed death in most reported cases to ensue in 6 to 12 months In 9 autopsy cases death was caused by either extensive pelvic recurrence or distant metastases

Incidence of primary malignant melanoma of the gastrointestinal tract excepting the anorectal canal is considered non existent but it is in fact due to the inability of diagnosis Metastatic gastrointestinal melanoma is no longer a medical curiosity (DAS GUPTA & BRASFIELD 1964 NATHANSON et coll 1967 WILLBANKS & FOGELMAN 1970 MENUCK & AMBERG 1975 FRASER MOODIE et coll 1976) In their report of 7 cases FRASER MOODIE et coll estimated small intestinal metastases to be far more common than realized Reporting 18 cases WILLBANKS & FOGELMAN quoted the slightly less than one per cent incidence of metastases (10 of over 1 000 skin melanomas) from the series of DAS GUPTA & BRASFIELD

There appear to be less than 200 cases of primary rectal malignant melanoma in the literature since the first description in 1857 STEARNS et coll reported 30 and PACK & OROPEZA reported 20 cases citing a 0 8 per cent incidence of all anorecto colonic malignancies HUSA & HOCKENSTEDT (1974) reviewed 4 206 cases of anorectal tumor and found a 0 33 per cent incidence of primary melanoma They attributed the uniformly poor prognosis to the stage of disease The type of surgery appeared to exert little or no influence on the prognosis

Primary malignant melanoma of the esophagus is even a rarer entity To date there are less than 40 confirmed cases in the western literature since the first case report in 1895 (RAVEN & DAWSON BASQUE et coll 1970 PICCONE et coll FRABLE et coll MUSHER & LINDNER KURZBAN et coll 1976 MÅNSSON & BERGE 1977) Of greater rarity is metastatic melanoma of the esophagus (WOOD & WOOD 1975) BUTLER et coll (1975) compiled 3 cases from the literature and they reported th fourth

Concurring with DAWSON (1925) and WILLIS (1948) FOWLER & SUTHERLAND (1952) postulated that esophageal melanoma might represent one of multicentric foci originating in the entire epi basal layers rendering permanent cure incon ceivable Histogenesis of esophageal melanoma was seriously questioned by STOUT & LATTES was not readily accepted as an entity until the 1960 s when melanin laden cells were identified the basal layers of the esophageal mucosa (DE L PAVA et coll RAVEN & DAWSON PICCONE et coll

Experience with esophageal melanoma has be starkly grim throughout the literature Half of th cases died of disease within 6 months with a average survival period of 7 4 months (BINGHA et coll) PICCONE et coll reported the lo recorded survival to be 15 months and the short 7 months

The value of radiation therapy for muco malignant melanomas other than oculo-conjunct presentation is difficult to assess but conventio radiation therapy with standard fractionati scheme is considered to be inferior to radi surgery Incurability of such tumor by radia therapy was stressed by COLLANTES et coll af reviewing 37 cases from the literature In th summary 4 of the 9 cases of vaginal melano treated with supervoltage irradiation and rad plaque died of wide spread metastases within a ye

Several case reports contain poor experience w immunochemotherapy for mucosal melanoma contrast to the encouraging outlook for sk melanoma (BLUMING et coll FRABLE et co MORTON et coll ALEXANDER & CONE 1977) T role of immunochemotherapy for mucosal me noma cannot be established with certainty at present time

In support of the widely held view of the futi of radiation therapy for malignant melanom HABERMALZ & FISCHER (1976) demonstrated striking correlation between the degree of respo and the size of individual radiation fraction Acco ing to their observation in none of 11 lesio treated with less than 5 Gy (500 rad) per fract even partial response occurred whereas in 18 of lesions given 6 to 23 Gy per fraction significan better response was obtained This observation to the conclusion that such a large size fraction required to overcome a large shoulder They e

ted this threshold fraction size to be in the ghborhood of 6 Gy They further concluded that re might be little or no correlation between the ponse probability and total radiation dose

The retrospective survey of the U K and lbourne data on malignant melanoma by HORN (1978) was also in agreement with their view porting the efficacy of a relatively few large ctions over many small fractions in local control melanoma Good tumor response was observed in to 58 per cent with the fraction size of 4 to 8 Gy $p<0.01$

In the present report is described one case each of mary malignant melanoma of the vagina and phagus treated by weekly or twice weekly large ction irradiation supplemented by protracted high se brachytherapy for long term local control

Case reports

Case 1 A 58 year-old female presented pigmented dular lesions in the vagina She was otherwise asymptotic except for vaginal discharge She underwent ab minal hysterectomy for benign uterine fibroid 13 years viously Examination revealed a bluish purplish raised dule of 1 5 cm diameter and 2 pin head sized flat ions in the mid third of the anterior vaginal mucosa pelvic or groin nodes were appreciated Examina n of the entire body surface skin revealed no pigmented in lesion Excisional biopsy showed malignant mela ma of deeply invasive type Nothing abnormal was und on chest films bipedal lymphangiography bone and er spleen scintigraphy nor at urethro-cystoscopy recto gmoidoscopy or CT of the pelvis and abdomen

Because of the tumor location and depth of invasion ginectomy pelvic and groin node dissection were con dered inevitable On the other hand the chance of mplete freedom from recurrence seemed small ac rding to the literature Under these circumstances it d not seem illogical to attempt a maximum local control irradiation

Eight weekly doses of 6 Gy via 2 wedge lateral and a ngle anterior pelvic ports with a 6 MeV linear accelera r were given The lesions regressed considerably in e ensuing 2 months

Three months later she underwent vaginal radium laque application which imparted 40 Gy to the mucosal rface In the following 6 weeks the lesion further gressed to measure 0 3 cm with faint colored pin head zed lesions

About 5 months after the beginning of treatment five 3 7 MBq (0 64 mCi) ^{125}I seeds were implanted to the base f the large nodule and one seed each to the pin head ized lesions A small composite isodose pocket of 300 Gy ver the total decay time period was confirmed by the omputer dosimetry

At this writing (10 months after the implantation) she remains free of local recurrence regional or distant metastases

Case 2 A 63 year-old male was admitted with a 6 week history of substernal discomfort left shoulder pain and weight loss A tumor was found in the middle of the esophagus below the level of the carina At operation it was found that the mediastinum the liver and celiac axis were free of metastases The tumor was removed and esophago-gastric anastomosis was carried out The sur gical specimen showed a 8 5 cm long variegated mass with a discrete 5 0 cm nodular mass in it Microscopically the main mass consisted of leiomyoma but the nodular mass represented malignant melanoma The melanomatous lesion was deeply invasive into the muscularis and the proximal margin of resection was transgressed by the tumor Scrutiny of the body surface skin showed no melanomatous lesion

He received 35 Gy to the esophageal surface mucosa by 6 twice weekly applications of a radium bouginage Following the second bouginage a 2 5 cm soft non tender node developed in the right supraclavicular region Excision of the node revealed metastatic malignant melanoma Six weekly doses of 5 Gy were given to the lower neck and upper mediastinum

He was controlled 2 years after beginning of treatment Scintigraphy of bone liver and spleen was then normal as well as chest film No local recurrence was found at radiography of the esophagus Clinically he remained in good health

Discussion

Primary malignant melanoma of the esophagus or vagina is extremely rare which is the cause that no therapeutic alternatives other than radical surgery have been exhaustively attested

It is doubtful that radical exenterative surgery can completely control a locally advanced mucosal melanoma Even if radical surgery can provide a better chance of local control the prospect of cure cannot be substantively improved because of the multicentric histogenesis and capricious metas tasizing behavior

While the value of immunochemotherapy is yet to be confirmed an appraisal of large fraction irradia tion seems to be appropriate

One case each of primary malignant melanoma of the esophagus and of the vagina are reported Both cases received non-conventional weekly or twice weekly large fraction/high dose protraction radiation therapy following excisional biopsy or wide excision alone of the primary or regional metastasis

Although long term observation is lacking a large fraction of 5 to 6 Gy can be repeated with impunity 6 to 8 times on a weekly or twice weekly basis No

acute morbidity or complication has arisen in either case

Such intermittent large fraction therapy may prove to be an effective adjunctive means in controlling a local mucosal melanoma

SUMMARY

Primary malignant melanoma of the vagina and esophagus are reported and the literature on the subject is extensively reviewed The role of large fraction irradiation is appraised In both cases no exenterative surgery was imposed but instead intermittent large fraction irradiation with protracted high doses was elected for local control

ACKNOWLEDGEMENTS

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FROM THE FONDATION BERGONIÉ CANCER CENTER F 33076 BORDEAUX FRANCE

HODGKIN S DISEASE CLINICAL STAGES I AND II

Results of radical irradiation with or without chemotherapy

B HŒRNI H EGHBALI M DURAND A DE MASCAREL D MAREE
G HŒRNI SIMON P RICHAUD J CHAUVERGNE and C LAGARDE

The introduction of lymphography in the staging Hodgkin s disease has individualized stages I and with lymph node involvement on only one side of : diaphragm In 1965 the Paris Symposium regu ed *radical radiation therapy for these stages* ereafter patients were submitted to this type of rapy The results show that about half of the tients were cured The failures can be attributed the fact that complete remission had not been duced or that patients had recurrent disease ese failures prompted the Stanford Group to per rm exploratory laparotomy with splenectomy in der to obtain *a more precise surgical staging and to* tend the field of irradiation in certain patients ADIN et coll 1971) Nevertheless even with sur cal staging the percentage of failures after en rged irradiation remained important Therefore it emed useful to attempt a management associating diation therapy and adjuvant chemotherapy ROSENBERG & KAPLAN 1975 ROSENBERG et coll 978) The drawback of this solution is that patients ith an overall good prognosis are submitted to sur ical staging and long heavy treatments which are ot without certain complications The authors are resently attempting to decrease the burden of this nedical management *which is not easy particularly* f laparotomy is to be avoided (BERNADOU et coll 78 Editorial Lancet II 1978)

The failures of radical irradiation used alone have prompted also other groups such as EORTC (1972) nd this institute to use adjauvant chemotherapy initially without surgical staging Previously prelimi nary results have been reported (LAGARDE et coll 1975) and now the results of 10 years of follow up are reported

Material and Methods

Between January 1965 and October 1976 190 patients with suggested Hodgkin s disease clinical stages I and II were treated at this institute His tologic slides were reviewed in 1976 for all cases and *the diagnosis of Hodgkin s disease was confirmed* for 177 patients only

Seven other patients were excluded from the pres ent analysis because they had not been irradiated 2 were treated before the Symposium of Paris held in February 1965 4 (between 69 and 85 years) because their general condition was poor due to age or another disease one refused treatment Six of these 7 patients died one is alive and disease free after secondary irradiation Thus 170 patients who had received radical irradiation according to the same procedures during the considered period remain for analysis

All these patients were seen at this institute at the time of diagnosis or soon after and none had been treated previously All diagnoses were established by biopsy and Hodgkin s disease was classified ac

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Table I

Main prognostic criteria

	Type of treatment		
	Irradiation alone	Chemotherapy + irradiation	Chemotherapy + irradiation + chemotherapy
Total	42	24	104
Male/female	21/21	13/11	71/33
Age			
<40	27	16	71
40–60	8	2	26
60	7	6	7
Histologic type			
I	5	5	30
II	20	13	50
III	15	3	23
IV	2	3	1
Clinical stage			
I	9	6	31
II	33	18	73
Mediastinal involvement	12	12	65
Infra-diaphragmatic disease	2	3	8
General symptoms (B)	5	8	26
Extranodal involvement (E)	1	3	11
Tumor diameter > 5 cm	7	10	55
Good prognosis	7	0	31

cording to international nomenclature (LUKES et coll 1966) All patients underwent clinical radiologic and biologic examinations according to the lines of the Rye Symposium (1966) with chest radiography tomography of the mediastinum bilateral lympho graphy from the foot and usual hematologic and biochemical blood tests No surgical staging pro cedures were performed diagnostic laparotomy was performed in 3 patients only All cases were thus classified in a clinical staging stage I one area in volved stage II two or more lymphatic areas in volved on only one side of the diaphragm The main prognostic criteria issued from these pretherapeutic examinations appear in Table 1 along with their distribution following the three treatment regimens On the whole 2 groups of patients are distinguished according to the data issued from the EORTC H1 trial (TUBIANA et coll 1979) (1) So-called patients with good prognosis fulfilling all the following cri teria less than 40 years old lymphoid predominance or nodular sclerosis at microscopy stages I or II with mediastinal involvement no systemic symptoms (A) no extra nodal (E) involvement (2) So called patients with poor prognosis with one or more of the *opposite criteria*

Treatment regimens were as follows Initially all patients were treated with radical irradiation only (R) *After having observed* many early failures with this treatment (1968) it was decided to submit patients with poor prognosis to an initial *chemotherapy followed* by the same radical ir radiation (C + R) Later on the treatment was rein forced as follows patients with particularly poor *prognosis (especially* those with bulky tumors) were given several courses of chemotherapy *before* ir radiation secondly patients in complete remission *received one* further course of *consolidation* chemotherapy lastly since this *sandwich* chemotherapy + irradiation + chemotherapy (C + R + C) *was* well tolerated and gave improved results in patients with poor prognosis it was *decided to* submit all patients to this combined therapy (*HŒRNI* et coll 1977) All treatments were given in a con trolled but not randomized manner *The details of* the different types of treatment given in each group of patients appear in Table 2

Table 2

Treatments

	Type of treatment		
	Irradiation alone	Chemotherapy + irradiation	Chemotherapy + irradiation + chemotherapy
Total number of patients	4[illegible]	[illegible]4	104
Irradiation			
Localized	5	1	5
Mantle	6	8	9
Mantle+lumbo-aortic	8	1[illegible]	82
Inverted Y	–	3	8
Splenic	0	0	0
Booster	1	1	3
Chemotherapy			
1 course before irradiation	0	24	93
–3 courses before irradiation	0	0	5
4–6 courses before irradiation	0	0	6
1 course after irradiation	0	0	100

Table 3

Results

	Type of treatment		
	Irradiation alone	Chemotherapy + irradiation	Chemotherapy + irradiation + chemotherapy
Total number of patients	42	24	104
Initial complete remissions	34	22	100
Initial failures	8	–	4
Relapses	14	7	5
Irradiated area	1	3	2
Marginal extension	3	1	3
Lymph nodes	9	1	1
Visceral	1	3	2
Persistent complete remissions	0	13	95

A few patients had two types of relapses

Almost all patients received irradiation with ^{60}Co at the dose of 35 Gy to 40 Gy in 3.5 to 4 weeks with a weekly dose of 10 Gy (2 Gy per day × 5 days). 40 Gy were given in the involved regions, 35 Gy in the adjacent areas. Because of 2 cases of myelitis during the first months and years following the treatment, after 1968 the spinal cord was protected with a 1.5 cm wide shield for the posterior field when the dose was higher than 20 Gy. It appears that this shield significantly decreases the dose absorbed by the spinal cord but the dose to the mediastinal lymph nodes is only slightly decreased. However this decrease has not yet been exactly calculated. Lumbo-aortic regions and mantle fields were more frequently irradiated in the C + R + C regimen (Table 2). Except for 4 elderly patients and one pregnant woman, mantle + lumbo-aortic regions were irradiated at the same time.

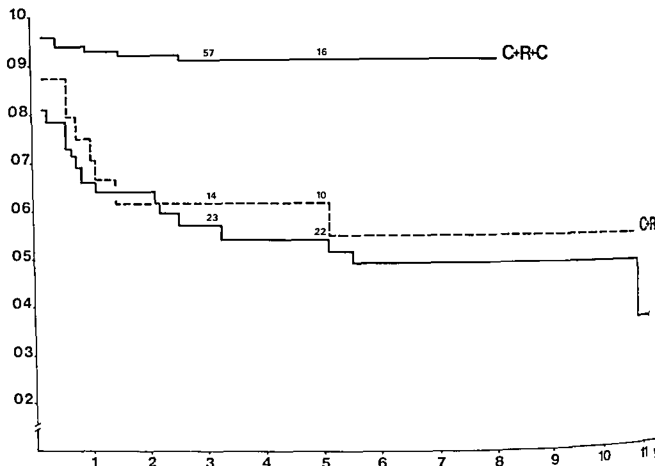


Fig 1 Actuarial recurrence free survival (a few patients in complete remission died of intercurrent disease) R = irradiation alone C + R = chemotherapy + irradiation C + R + chemotherapy + irradiation + chemotherapy

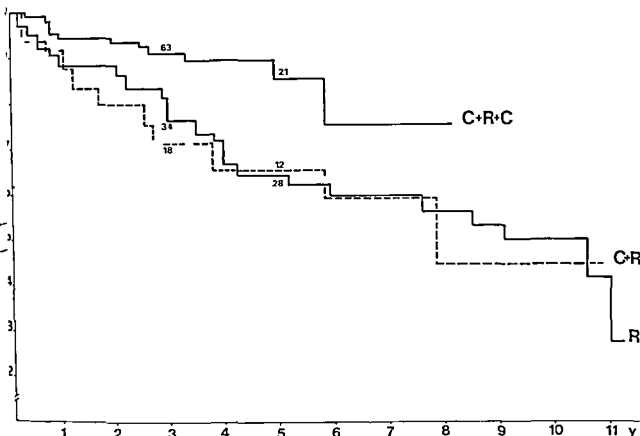
Chemotherapy was usually an association of cyclophosphamide (200 mg every other day) + vinblastine (10 mg per week) + procarbazine (300 mg per day) + methylprednisolone given according to a previously published schedule (CHAUVERGNE et coll 1973) the treatment was discontinued as soon as the patient s leukocyte count dropped to 2×10^9 l usually before the 21st day thus the treatment was usually shorter for the consolidation course than for the induction course Most patients submitted to this schedule required hospitalization Patients with bulky tumors who had received several courses of chemotherapy did not receive the treatment mentioned which is too poorly tolerated when repeated more than two times, they received the well known MOPP schedule (DE VITA et coll 1970)

Radiation therapy was initiated in all patients immediately after the end of the initial 15 to 20 day course of chemotherapy A rest period of one month was inserted between the end of irradiation and the beginning of the consolidation chemotherapy the average duration of this rest period was 33 days (for 5 patients chemotherapy could not be repeated two months later as the leukocyte count was satisfactory after only a one month rest period)

No maintenance treatment was given after radiation or consolidation chemotherapy administered as described Thus the overall treatment brief one month when irradiation was given (R) 6 to 7 weeks when chemotherapy + irradiation were administered (C + R) about 3 months patients given chemotherapy + irradiation + chemotherapy (C+R+C)

In case of relapse patients were restaged treated according to the best currently available treatment

Except for one patient who was lost to follow all were followed up until their death or until the months of 1978 The follow up period is thus than one year for all patients All data were collected during May 1978 They were transcribed on a standard form for each patient Survivals were calculated according to the actuarial method and significance according to the log rank test (PET



2 Actuarial crude survival Symbols as in Fig 1

ll 1977) Crude survival is considered from the ıe of diagnosis Disease free survival is con lered from the last day of radiation therapy

Results

Complete remission was obtained in 34 of 42 tients given irradiation alone and in 122 of 128 itients given initial chemotherapy followed by ir diation This significant difference (p < 0 01) ap ars in Fig 1 along with the actuarial curve of omplete remission or recurrence free survival (a w patients in complete remission died of intercur nt disease) This figure shows that patients given radiation alone have a plateau after 6 years (except or one patient who relapsed at 11 years) The isease free survival of so called patients with poor rognosis initially given chemotherapy followed by radiation is quite the same The curve of patients eated with a sandwich schedule (C + R + C) is ery different no relapse occurred beyond 3 years nd 57 patients have exceeded this period the plateau is above 90 per cent (at 0 9087 exactly) it can thus be expected that 90 per cent of these patients are cured

The types of relapses are shown in Table 3 All types of relapses are reduced in patients given com bined treatments however relapses in distant lymph nodes are particularly frequent in patients treated with irradiation alone The decrease in this latter type of relapse may be due to the fact that the lumbo aortic area was irradiated more often in the combined regimen If patients with good prognosis and poor prognosis are considered separately in the first group 2 failures of 7 R patients occurred and none of the 31 C + R + C patients in the second group 21 failures of 35 R patients 9 failures of 24 C + R patients and 9 failures of 73 C + R + C patients occurred

A few patients who did not respond or who re lapsed obtained complete remission by another treatment and remained in complete remission with a follow up of 2 years or more 4 of 22 failure R patients 1 of 9 C + R patients and 3 of 9 C + R + C patients Thus it appears that it is rare to cure a patient after a primary failure or a relapse

Table 4

Course and survival

	Type of treatment		
	Irradiation alone	*Chemotherapy + irradiation*	*Chemotherapy + irradiation + chemotherapy*
Total number of patients	42	24	104
Total cumulative follow up (months)	*3 139*	*1 364*	*4 587*
Intercurrent diseases			
Herpes zoster	7	6	21
Other infections	1	1	9
Therapeutic complications	5	1*	6
Second malignancy	2	1	6
Hemolytic anemia	1	1	1
Miscellaneous	3	0	7
Normal pregnancies	*5*	*0*	*4*
Deaths			
from Hodgkin s disease	17	6	5
Therapeutic complication	[illegible]	1	1
Second malignancy	2	0	4
Other	2	2	[illegible]

? myelitis 3 fibrosis of brachial plexus 1 hypothyroidism
Femoral osteonecrosis
5 transitory bone marrow aplasia 1 intestinal complication 1 pneumothorax

Survivals appear in Fig 2 These are uncorrected survivals including all causes of death (which are detailed in Table 4) These results are poorer than those for recurrence free survivals About three fourths of the patients (28/40) with primary failures or relapses died this proportion is slightly lower in patients who had been treated with a combined treatment (11/18) than in those who had been initial ly treated with irradiation alone (17/22) however this could be due to a shorter follow up period for patients given chemotherapy It is striking that while Hodgkin s disease is the first cause of death in patients given irradiation alone (17/23) it accounts for only 5 of 12 deaths in patients who received sandwich treatment Among other causes of death a second malignancy has a prominent place

No immediate serious complication due to treat ment was observed The detailed hematologic to lerance of the sandwich regimen was presented elsewhere (EGHBALI et coll 1978) Some severe de layed and eventually lethal complications were ob served in the three groups of patients (Table 3) In 2 patients myelitis occurred after irradiation per formed without protection of the spinal cord (Idl RY et coll 1971) In the sandwich regimen 5 patie developed a severe bone marrow aplasia at the e of the last course of chemotherapy but they covered without complication (2 of them wi h antibiotics) within 10 days

Table 4 shows other pathologic conditions served in these patients Herpes zoster was the m frequent infection however it was not more f quent in patients given chemotherapy than patients without chemotherapy (AUBERTIN et c 1978) On the contrary other infections seemed be more frequent in the third group of patients this may be due to a longer period of follow-up only infection related to management of Hodgki disease was a pneumococcal septicemia superven in a 15 year-old boy who had undergone laparoto and splenectomy because of suggested relapse years after initial treatment Out of the therape complications 4 were lethal (2 post irradiati myelitis 1 bone marrow aplasia following the tre ment of a relapse and the septicemia mentioned second malignancy was observed 9 times in

Table 5

Nine secondary malignancies

Type of treatment	Type of malignancy	Delay between treatment and second malignancy	Localization in an irradiated region	Sex and age
Irradiation alone	Esophageal carcinoma	9¼ years	Yes	F 36
	Esophageal carcinoma	8 years	Yes	M 74
Chemotherapy + irradiation	Skin carcinoma	2½ years	No	F 77
Chemotherapy + irradiation + chemotherapy	Acute myeloblastic leukemia	5½ years	—	M 35
	Laryngeal carcinoma	2 years	No	M 54
	Bronchial carcinoma	3 years	Yes	
	Osteogenic sarcoma	5 years	Yes	M 18
	Ovarian immunoblastosarcoma	8 months	No	F 19
	Bronchial small cell carcinoma	2 years	Yes	M 26

tients 5 carcinomas seem to be unrelated to atment one esophageal in an elderly alcoholic tient one double (larynx + lung) in a heavy ioker one skin carcinoma in an elderly patient d one immuno blastosarcoma supervening only 8 onths after the initial diagnosis of Hodgkin s dis se (Table 5) Such secondary non Hodgkin s lym omas were recently reported but always after an erval of over 5 years (KRIKORIAN et coll 1979) ie other malignant tumors may result from the atment given for Hodgkin s disease they were ore frequent in the third group of patients Three alignant tumors may be related to the treatment nong the 104 C + R + C patients or more ac irately among the 95 patients apparently cured om Hodgkin s disease after C + R + C treatment o of them occurred in an irradiated area (bronchial ircinoma and clavicle osteosarcoma)

Discussion

It is difficult to compare the results obtained with ie three therapeutic regimens since they were not andomized Moreover patients had been allocated the second regimen (C + R) because of the pres nce of poor prognosis criteria The first group of atients received irradiation alone and the results in his group are comparable to results obtained in ther somewhat old series of patients who had been staged only by radiologic and clinical procedures and irradiated in the same manner (EASSON 1966 KAPLAN 1966) It is more difficult to compare the results of the C + R + C regimen with other recent series because most of these series include patients after surgical staging However the results obtained with this regimen seem valuable and should thus be considered when the best therapy in a similar type of patients is discussed Similar results were obtained without laparotomy and extended radiation therapy but only for patients with clinical stage I A and II A (GRIFFIN et coll 1977) or with a longer period of chemotherapy (ANDRIEU et coll 1979)

The C + R + C regimen is fairly well tolerated considering it is somewhat heavy Hospitalization is usually required during chemotherapy but the over all treatment is quite brief Large mantle and lum bo aortic irradiation can be given at the same time immediately after the first course of chemotherapy without any major complication The hematologic effects of this regimen have been detailed elsewhere (EGHBALI et coll) No deaths directly related to the initial treatment occurred The only serious com plication is second malignancy observed after as sociation of chemotherapy and irradiation as noted in other series (ARSENEAU et coll 1977 BRODY et coll 1977) In comparison with other series the overall management is also lightened since neither surgical staging nor maintenance chemotherapy are needed Moreover the spleens of these patients

were not surgically removed or irradiated and no unfavorable consequences were observed

Thus it seems difficult to improve the results About 5 per cent (6/128) of the patients were initially resistant to treatment (both chemotherapy and radiation therapy) and were unable to obtain com plete remission Moreover 5 per cent of the patients (5/104) relapsed since relapses are varied it is dif ficult to propose any preventive therapeutic measure Only 4 of 18 patients initially treated by C +R or C+R+C with initial or secondary failures had been treated subsequently more heavily and are now without evidence of disease and apparently cured Therefore it must be pointed out that these rather good results are obtained with a heavy but brief treatment which does not require surgical stag ing removal or irradiation of the spleen or long term chemotherapy

However second malignancies appear with a low but threatening frequency Some of them are cer tainly favored by the association of chemotherapy and irradiation It is therefore necessary to try to alleviate the therapeutic regimen It has been sug gested that the radiation doses could by reduced after chemotherapy 15 to 20 Gy might be sufficient (PROSNITZ 1976) The suppression of chemotherapy would be particularly useful in young male patients because this treatment is often responsible for azoospermia (CHAPMAN et coll 1979) This en deavor is necessary at least in patients with good prognosis In this latter group no failures occurred in 31 patients given the C + R + C treatment and very few failures in other patients more lightly treated However even in this category of patients with good prognosis the percentage of relapses observed in the H1 trial after radical irradiation with or with out vinblastine maintenance chemotherapy was slightly more than 25 per cent (TUBIANA et coll) There are reasons for reluctance in exposing patients to relapse since the possibilities of cure after relapse although not negligible are small (WELLER et coll 1976 DE VITA 1977) One way is to submit patients with good prognosis to exploratory laparotomy so that a more accurate prognostic evaluation could be obtained this is currently being done by the Radiotherapy-Chemotherapy Group of the EORTC Another way is rather to reduce the irradiated fields such a randomized trial is now un derway in the Pierre-et Marie Curie Group in France

In summary the results show that it is possible to give these patients a heavy but short treatme without surgical staging and splenectomy or splen irradiation and without long term chemothera and to obtain about 90 per cent of cure Howev the treatment is still to be lightened mainly fo patients with good prognosis in order to reduce th occurrence of complications mainly second mal nancy

SUMMARY

From 1965 to 1976 170 patients with Hodgkin s disea clinical stages I or II were treated with radical irradiatio A first group of 42 patients was treated with irradiati alone a second group of 24 patients received one cour of chemotherapy followed by irradiation and a third gr of 104 patients was treated with a sandwich regimen in cluding chemotherapy + irradiation + chemotherapy Al though the treatments were not randomized the com parison of the results shows a striking improvement in the group submitted to the C + R + C regimen in this gro the plateau of disease free survival was 90 per cent Th result was obtained after treatment for about 3 mo without surgical staging or maintenance chemothera However this treatment may favor some complicatio mainly the development of a second malignancy the risks may be decreased by reducing the treatmen patients with good prognosis

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CHLORAMPHENICOL TOXICITY IN RADIATION DISEASE

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J NETIKOVA and S VIKLICKA

In view of the role of bacteremia or bacterial temia in the pathogenesis of lethality following irdiation antibiotics range among significant means a complex therapy of the radiation disease However drugs which might damage the bone marrow d thus aggravate the radiation induced depression the hemopoietic functions are contraindicated iese drugs may also include chloramphenicol AP) which is a potentially myelotoxic agent UNIS 1969) It has been the aim of the experiments w reported to confirm the possibility of a risk sociated with CAP application in the radiation dis se Care has been taken to choose such experi ntal conditions that would correspond to high rapeutic doses of the antibiotic and its adminis ition at a time when the protective abilities of the ganism have declined (CRONKITE & BOND 1960) ie experiments have been conducted on mice nployed for analysing the CAP hematotoxicity IRKIN et coll 1974)

Material and Methods

Male mice of the $C_{57}Bl/10$ strain were used The imals were irradiated at the age of 12 to 13 weeks an average body weight of 25 g Standardized diet nd tap water were given ad libitum The mice were radiated with a TUR apparatus operated at 180 kV mA filtration 0 5 mm Cu and 0 5 mm Al dose te 0 7 Gy/min Single whole body doses of 4 8 Gy nd 6 2 Gy were given in the morning

CAP–chloramphenicol soluble (...) SPOFA (chloramphenicolum succinicum natricum) was injected subcutaneously every eight hours in doses of 320 mg of base per 1 kg of body weight in volumes of 0 2 ml Control animals were injected equivalent volumes of water for injection A 5 day (15 injections the last one given in the evening) and 3-day treatment (10 injections the last one given in the morning) were chosen of which the former was started 5 days the latter 7 days after irradiation In normal unirradiated mice the serum levels of active CAP were determined by the method of LEVINE & FISCHBACH (1951) 4 hours after the first subcutaneous dose of 320 mg/kg of body weight An average serum concentration of 14 6 µg/ml was found

Determination of peripheral blood cells Blood samples were drawn from a fine incision in the tail vein The total red and white blood cell counts were determined using a Coulter Counter apparatus Repeated blood withdrawals were made which made it possible to express changes in individual values with respect to the initial state

^{59}Fe incorporation into the heme of erythropoietic organs ^{59}Fe citrate (Rotop G D R) diluted with physiologic saline with the addition of benzyl alcohol 0 9% and i p injected in an amount of 3 7×10^4 Bq was used After 6 h the animals were killed by cervical dislocation the spleens were excised and the skeletons were cleared of the soft tissues by placing into a colony of the saprophagic insect Der

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mestes vulpinus Fbr. Extraction of heme iron from the spleen and the entire skeleton was carried out by an original method (VACHA et coll. 1978) with the aid of acid ethylacetate. Radioactivity of the extracts was measured using the Nuclear Chicago Automatic Gamma Well Counting System and expressed as per cent of the activity applied.

Bone marrow cellularity. Bone marrow of femoral diaphyses was washed with isologous serum and suspended. From a part of the suspension a smear was prepared which was differentiated after staining with May-Grunwald and Giemsa-Romanowski stains. The rest of the suspension served for determination of the number of nucleated cells in Burker's chamber. Erythropoiesis was represented by proerythroblasts and by basophilic, polychromatophilic and orthochromatic erythroblasts, granulopoiesis included all mature and immature forms of eosinophilic, basophilic and neutrophilic granulocytes. Nucleated elements included cells of the erythroid and granulocytic line and moreover lymphocytes, megakaryocytes, plasma cells, monocytes, reticular cells and unidentifiable cells.

Spleen colony forming cells (CFU-S). The method of TILL & MCCULLOCH (1961) was used. A pool of tibial bone marrow cells was prepared by standard procedure and the recipients injected different amounts (3×10^4 to 2×10^5) of cells 3 h after irradiation with 6.2 Gy. On day 10 after irradiation the surface splenic colonies were counted after fixation in Bouin's solution. After subtracting the background of endogenous colonies, the number of colonies per bone marrow in one tibia was determined.

Autoradiography. Bone marrow of the femurs was treated by standard procedure (BENEŠ & DRÁŠIL 1962). Cell suspensions were labelled with 7.4×10^4 Bq ^{3}H-dTh (per ca 10^7 cells) at 37°C for 1 hour. Autoradiography using the stripping film technique (Kodak AR 10) was carried out from the smears. The labelling index (number of labelled cells per 1 000 observed cells) characterizing the number of cells in S phase of the cell cycle was evaluated. A labelled cell was determined with 99 per cent probability. Labelled cells of the red line are represented by proerythroblasts, basophilic and polychromatophilic erythroblasts, granulopoiesis included myeloblasts, promyelocytes and myelocytes.

Statistics. The Student's t test and the chi square test were used. The values given in the data represent the mean ± SE.

Table 1

^{59}Fe incorporation into heme of spleen and bone marrow ... whole skeleton in control and CAP-treated mice 10 and 2... after 4.8 Gy (6–8 animals per group used)

	10 days after irradiation		0 days after irradiation	
	Spleen	Bone marrow	Spleen	Bone marrow
4.8 Gy	0.84±0.1[illegible]	7.00±0.64	18.04+1.[illegible]	7.6[illegible]
4.8 Gy+CAP	0.[illegible]±0.05	3.00±0.36	17.80±1.64	[illegible]

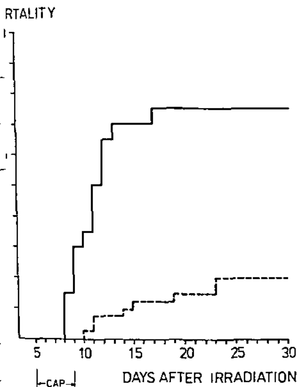
* Difference from control $p<0.01$

Results

An obvious evidence of CAP toxicity in the i... radiated organism is provided by the rate of survi... of irradiated animals (Fig. 1). A 5-day CAP tr... ment resulted in a significant ($p<0.01$) increase... the 30-day mortality after 6.2 Gy (75% compar... with 20% in controls). No mortality occurred in a... mals irradiated with 4.8 Gy and treated with t... employed CAP doses. Effects of CAP on hem... poiesis were further examined using this sublet... dose.

Results given in Table 1 demonstrate a depressi... influence of the 5-day treatment with CAP upon ^{59}... incorporation into the heme of spleen and bone m... row at the end of the treatment period, i.e. 10 d... after irradiation. Twenty days after irradiation ... ferences were no longer observed, which sugge... reparability of CAP-induced depression of the he... synthesis. Whereas ^{59}Fe incorporation into the he... of the bone marrow remained at this postirradiat... period approximately on a normal level, ^{59}Fe in... poration into the heme of the spleen exhibited a b... overshoot (the normal level of ^{59}Fe incorpora... into splenic heme amounting to about 2.1%) co... parable to the findings on the dynamics of pos... radiation recovery of erythropoiesis (BRADY et c... 1976).

Peripheral leukocyte and erythrocyte counts w... determined in irradiated animals after a 3-day C... treatment. The effects of CAP on erythro... counts are shown in Fig. 2 as per cent change aga... the values determined on day 6 after irradiation ... before starting CAP treatment. A transitory ... crease in peripheral erythrocyte counts is appar... in the CAP-treated group on day 14 after irradiati...



1 Cumulative mortality (in per cent) in 40 control (—) and 20 ...treated ( ) mice after 6 2 Gy

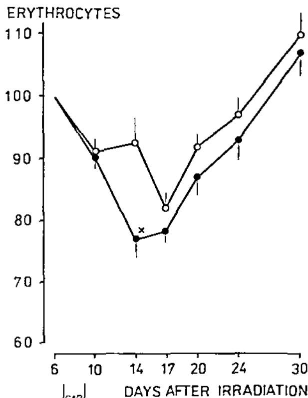


Fig 2 Per cent of erythrocyte counts in control (O) and CAP treated (●) mice at various intervals after 4 8 Gy (10 animals per point used × difference from controls p<0 05)

...ate of recovery beginning 17 days after irradia... ...oes not seem to be affected The leukocyte ... in the same experiment are given in Fig 3 ...esults are expressed by means of a factor of ...ise i e the ratio of leukocyte counts at a given ...al to the leukocyte counts before CAP treat... ...on day 6 after irradiation (average value 860 ...mm³) In the CAP treated animals a lower rate ...ikocyte count recovery is obvious which is ...ted by decreased values observed on day 10 after irradiation and by a lower level of leukocyte counts at other intervals of postirradiation recovery

A 3 day CAP treatment was chosen to analyse the effect of the antibiotic on the cytology and content of CFU S in the bone marrow of normal and irradiated mice Bone marrow samples were drawn 4 h following the last injection of CAP that is 10 days after 4 8 Gy in experiments with irradiated mice As expected 10 days after irradiation bone marrow cellularity and number of CFU S were decreased (Ta...

Table 2

Number of cells of the main bone marrow populations (in thousands per femur) and of CFU S per tibia in non irradiated and irradiated (10 days after 4 8 Gy) control and CAP treated mice (for the graphic representation of the differential count 3 animals per group for CFU S 5 donors and 10 recipients per group used)

	Non irradiated	Non irradiated + CAP	4 8 Gy	4 8 Gy + CAP
Erythropoiesis (total)	748±463	945±..1	465± 67	244±65
Granulocytopoiesis (total)	? 976±168	1 507±85	365±168	252±89
Lymphocytes	939±3 4	? 06?± 00	.59±101	186±45
Nucleated elements	10 567±775	6 400±551	1 983±60	1 467±394
CFU S	1 3 3±48	909±43	?.7±.1	50±6

Difference from controls p<0 01

ble 2) The total cellularity as well as cells of the erythroid granulopoietic and lymphoid lines were decreased after CAP treatment both in control and irradiated animals Due to the small number of mice used no statistic evaluation of these results was made The amount of CFU S in the tibia was how ever significantly decreased in the CAP treated groups and reached its lowest level with the ir radiated and CAP treated animals

Analogous conditions of 3 day CAP treatment were employed to analyse cytokinetic characteris tics of femoral bone marrow by autoradiography (Table 3) Irradiation per se reduces in a given interval the number of white and red blood cells in the S phase However the differences are not statis tically significant A recovery of postirradiation mitotic depression can already be anticipated 10 days after sublethal irradiation However CAP de creased significantly cellular proliferation in both unirradiated and irradiated animals in either case more markedly with the erythroid line The numbers of marrow cells entering S phase were lowest with the irradiated and CAP treated group

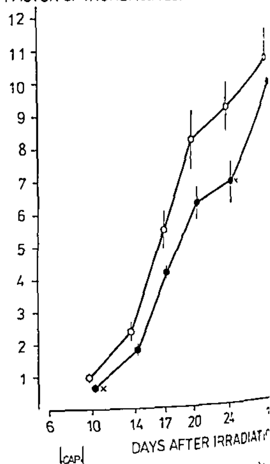


Fig 3 Factor of increase f leukocyte counts in control (CAP treated (●) mice at various intervals after 4 8 Gy (10 a per point used × difference from controls p<0 05)

Discussion

The CAP dose used corresponds with respect to the serum concentration achieved to clini cally employed doses designated as high ones (BARTMANN 1974) According to SUHRLAND & WEISBERGER (1963) erythropoietic disorders are to be anticipated when serum concentrations of CAP higher than 15 μg/ml are reached These calculations do not take into consideration possible changes of the pharmacokinetics of CAP in the irradiated or ganism A significant affection of the liver and kid ney functions which might lead to persistence in the blood of higher CAP values and thus to more marked manifestations of its toxicity (SUHRLAND & WEISBERGER) cannot however be expected under the acute conditions following the radiation doses used

The finding of an increased postirradiation lethali ty after CAP treatment may be considered as the result of an additive action of radiation and CAP on the hemopoietic functions The action of CAP is manifested by a transitorily lower effectiveness of production of the red and white blood elements and its cause is evidently also the depressive action of CAP at the level of the stem cells

FIRKIN et coll injected normal mice with higher

Table 3

Number of femoral cells in S phase in n irradiated and irradiated (10 days after 4 8 G control and CAP treated mice as per cent of n irradiated control group (5–10 autoradiograph evaluations per group used)

	Non irradiated	4 8 Gy
Controls		
Erythropoiesis	100	71 0
Granulocytopoiesis	100	83 8
CAP treated		
Erythropoiesis	41 8*	37 4
Granulocytopoiesis	74 7	67 5

Difference from controls

Difference between erythropoiesis and granu locytopoiesis p<0 01

P doses maintaining blood levels of 20 to 40 μg/ for 5 days and observed reduction of reticulo es in the blood decreased numbers of nucleated s in the marrow and an increase in splenic col forming units (CFU S) and in the agar colony ming cells (CFU C) This effect was explained by nulation of CFU S and CFU C in the bone mar with a lowered degree of their differentiation mature cell forms The disagreement of the sent results with those of FIRKIN et coll may h respect to CFU S behaviour be caused by ering experimental conditions and by differences dynamics and distribution of stem cell compart nts in various strains of mice An inhibitory ac n of CAP in therapeutic doses upon the growth of man and mouse marrow cells in agar cultures was nonstrated by MORLEY et coll (1974) With the of techniques for in vitro examination of the wth of mouse and human erythroid (CFU E) and nulocytic (CFU C) colony forming units YUNIS ADAMSON (1977) demonstrated increased vulner lity of the erythroid stem cells to CAP In agree nt with these findings are also the present results owing a more marked inhibition of proliferation of rrow cells of the red line during CAP treatment in o These effects are apparently related with the nical experience that suppression of erythro iesis from large doses of CAP occurs with much ater regularity than suppression of granulo iesis (YUNIS)

The suppression of hemopoiesis by CAP probably sults from the inhibition of mitochondrial protein nthesis (MARTELO et coll 1969 NIJHOF et coll 77) which must ultimately be reflected also in ppression of cellular proliferation and differentia n Particularly the rapidly dividing cells and tis es are thus prone to side effects of this antibiotic vivo The results presented show that these ef ts may adversely affect recovery processes in mopoietic cell populations of the irradiated or nism and corroborate the assumption that radia n syndrome is a contraindication for the thera utical use of CAP

SUMMARY

The effect of chloramphenicol given in high therapeutic ses in the course of postirradiation syndrome in irradiated mice of C $_7$Bl/10 strain is reported Chloram phenicol treatment increased postirradiation mortality of animals and intensified suppression of the proliferating and differentiating processes in hemopoietic organs of the irradiated animals The results support the assumption that postirradiation depression of hemopoiesis is a con traindication to the therapeutic employment of this an tibiotic

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COLLIMATION OF HIGH ENERGY ELECTRON BEAMS

I LAX and A BRAHME

The principal aim in the design of high energy ctron beams for radiation therapy is to obtain oad uniform beams of well defined energy and rection Of equal importance is the collimation of e beam in order to limit its size and to protect rrounding healthy tissues Even if the electron am flattening system is almost ideal producing a ane parallel and monoenergetic beam the colli ator will necessarily degrade the quality of the am to some extent This degradation will be eater for smaller field sizes since the fraction of llimator scattered electrons becomes of increasing iportance Therefore the purpose was to optimize e collimation geometry and the choice of col nator materials in order to obtain a minimum ntribution of scattered electrons in the therapeutic am (BRAHME 1978)

Several reports on the effect of electron scatter g near a discontinuity have appeared due to its iportance in dose planning (BREITLING & VOGEL 63 KARJALAINEN et coll 1968 POHLIT 1969 ANDOUR & HARDER 1978 GOITEIN 1978) How er no systematic analysis of the electron scatter g at a collimator edge where electrons are com etely stopped seems to have been published This oblem has particular implications in the use of nall field sizes since electrons scattered from the ollimator can contribute considerably to the ab- orbed dose and perturb the dose distribution of a am of otherwise high quality i e of small energy id angular spread

The field size dependence of the central axis pth dose distribution has been investigated ex nsively (MARKUS 1960 V D DECKEN 1956) The reduced build up and loss of therapeutic range for small field sizes is due to two different effects which previously have not been clearly distin guished experimentally It will be demonstrated that the influences of both these effects namely that of the collimator scattered electrons and that of the field size itself on the dose distribution depends on the lateral motion of electrons in the materials of the collimator and the phantom respectively

Theory

General considerations Four principal phe nomena are involved when broad electron beams are collimated in order to produce therapeutic electron beams of highest possible quality and consequently smallest possible beam contamination

First the presence of air is of great importance particularly at energies below 15 to 20 MeV since it greatly influences the paths of the electrons due to the appreciable scattering power of the air at these energies (Fig 1 ray No 1) This problem has been treated in detail by BRAHME (1971 1977) and it can conveniently be overcome by the balloon type of collimation

Secondly the collimator wall which ideally should be parallel to the direction of the electron paths in the beam will generally scatter slightly degraded electrons back into the beam (BRIOT et coll 1973 Fig 1 ray No 2) This phenomenon has been analysed extensively by MANDOUR & HARDER (1975) and MANDOUR (1978) It is of less

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importance when the collimator and the effective electron source are well aligned and the effective source size is small so that the electrons reflected almost at glancing incidence will lose very little energy remaining almost indistinguishable from the primary beam

However when the collimator walls are not parallel to the electron paths they may contribute considerably to the angular spread of the electrons reaching the irradiated surface (BRAHME 1978) This will increase the absorbed dose at the surface and displace the point of maximum absorbed dose closer to the surface since the electrons more rapidly reach a state of full diffusion (BRAHME 1978) This effect has recently been observed experimentally (VAN DER LAARSE et coll 1978) It is only to a small degree due to the energy degradation of the electrons scattered from the collimator (cf MANDOUR & HARDER 1975)

The third type of contamination is due to the production of bremsstrahlung in collimators or apertures (Fig 1 ray No 3) and has also been treated extensively (WIDEROE 1959 GIARRATANO et coll 1975) In principle it can be overcome by using low atomic number collimators followed by high atomic number photon absorbers

The last type of contamination which will be reported here in more detail is often of the greatest importance for the dose distribution particularly in the build up region This contamination is due to the large number of electrons which enter the collimator on the source side and are not stopped but scattered out into the beam through the collimator edge (Fig 1 ray No 4)

Angular distribution It has been shown that in the Gaussian approximation the spherical fluence of primary electrons at a depth z in a medium is according to BRAHME (1975) given by

$$\Phi(\theta_x, z)=\frac{\exp\{\theta_x/\overline{\theta^2}\}}{(\pi\overline{\theta^2})^{1/2}\cos\theta_x} \qquad (1)$$

where θ_x is the projected angle of the electrons on the $x-z$ plane and $\overline{\theta_z^2}$ is the mean square scattering angle of the electrons at a depth z (Fig 2) From the spherical fluence the plane fluence in a direction orthogonal to the direction of the incident beam for example in the positive x direction can be calculated

$$N_x(z)=\int_0^\infty \Phi(\theta_x, z)\sin\theta_x \, d\theta_x \qquad (2)$$

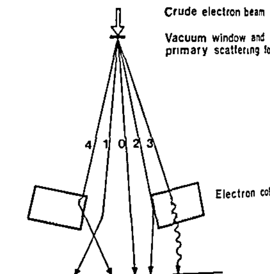


Fig 1 Representation of the 4 different types of first or... contaminations in therapeutic electron beams Ray No 1 represents an air scattered electron No 2 an electron sca... at the collimator edge No 3 an interaction in the coll... where a bremsstrahlung photon is produced and No 4 a sca... ing process in the collimator where the electron is scatt... out through the collimator edge

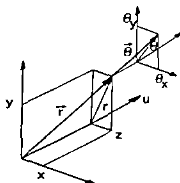


Fig 2 Coordinate system used to describe the lateral ... angular distribution of electrons in an electron beam

If the mean square scattering angle $\overline{\theta^2}$ is less than ... the integral in eq (2) can be developed in a pow... series When this is the case the unidirectional ... plane fluence along the x axis takes the form

$$N_x(z)=\left(\frac{\overline{\theta_z^2}}{4\pi}\right)^{1/2}\left\{1+\tfrac{1}{3}\overline{\theta^2}+\tfrac{1}{4}\left(\overline{\theta^2}\right)^2+\ldots\right\} \qquad (3)$$

The mean square angle of scattering at the dep... z is according to BRAHME (1975) given by

$$\overline{\theta^2}=\overline{\theta_0^2}+T_z\left(1-\frac{S_{col}}{E_p}z\right)^{-1} \qquad (4)$$

where $\overline{\theta_0^2}$ is the mean square angle of the incid... beam at $z=0$ T is the linear scattering p...

Table 1

The diffusion depth z_d/mm for different materials and energies

Material	H_2O	CH_2	C	Al	Cu	Sn	W	Pb	U
Ep/MeV									
5	15.2	16.3	10.9	3.4	0.97	0.85	0.24	0.38	0.21
10	34.0	35.6	23.6	17.0	2.48	2.26	0.66	1.05	0.60
20	68.9	71.4	46.8	24.7	5.37	5.06	1.52	2.43	1.40
30	98.3	102.1	66.4	34.7	7.50	7.06	2.17	3.46	2.00
40	123.0	[illegible]	82.7	47.4	8.98	8.36	2.56	4.14	2.39
ϱ/g cm^{-3}	1	1.05	1.73	2.69	8.92	7.31	19.3	11.3	18.7
D/D_{H_2O} eq (16) 10 MeV	1.00	0.95	0.66	0.44	0.13 Brass 0.14	0.14	0.047	0.081	0.048
D/D_{H_2O} experimental 10.5 MeV		0.95		0.44	0.16		0.05	0.09	

hich is obtained by multiplying the mass scatter ig power with the density (BRAHME 1972 ICRU lo 21 1972)

The factor within the parentheses (which corrects or the increase in scattering power with increasing epth) contains the total stopping power S_{tot} of the nedium and the most probable electron energy The mean unidirectional plane fluence along the x xis over the depth interval of the practical range R_p can be evaluated by integrating eq (3) after in ertion of eq (4)

$$N_x = \frac{\int_0^R N_x(z)\,dz}{R_p} \approx \left(\frac{TR_p}{9\pi}\right)^{1/2} \left\{1+\frac{\overline{\theta_0^2}}{TR_p}\left(\frac{3}{2}-\overline{\theta_0^2}^{1/2}\right)+ \quad \right\} \quad (5)$$

n order to analyse the material and energy de endence of eq (5) a first approximation may be ssumed that $R_p = kE_p$ with k=0.5 g cm^{-2} MeV^{-1} ndependent of material and $T=(E_s/E_p)^2 X_0^{-1}$ where E_s=21.2 MeV and X_0 is the radiation length ac ording to ROSSI (1952) If it is further assumed that $\overline{\theta_0^2}$=0 eq (5) appears as

$$N_x \approx \left(\frac{kE_s^2}{9\pi}\right)^{1/2} \times (E_p X_0)^{-1/2} \quad (6)$$

Thus the mean unidirectional electron fluence in a narrow Gaussian beam decreases with energy but ncreases with atomic number since the radiation ength is inversely proportional to the atomic num ber It should be pointed out that the eqs (5) and (6) are slight overestimations since the angular spread remains approximately constant at depths beyond the diffusion depth (cf eq 13)

Energy distribution Since the electrons are scattered more and more laterally at increasing depths the mean energy of the electrons leaving the collimator edge is considerably lower than the in cident energy If it is assumed that the mean energy of the electrons decreases linearly with depth ac cording to $E(z)=E_0[1-(z/R_p)]$ the mean energy of the laterally scattered electrons $\overline{\overline{E}}$ can be obtained by evaluation of

$$\overline{\overline{E}} = \frac{\int_0^R E(z) N_x(z)\,dz}{\int_0^R N_x(z)\,dz} \quad (7)$$

which after insertion of eq (3) reduces to

$$\overline{\overline{E}} = 0.4\, E_0 \left\{1 + \frac{1}{4}\left(\frac{\overline{\theta_0^2}}{TR_p}\right) + \quad \right\} \quad (8)$$

The mean energy of the laterally scattered electrons is thus approximately 40 per cent of the incident mean energy regardless of the material when the mean square scattering angle of the incident elec trons is small

Lateral electron displacement In order to obtain a complete description of the total number of low energy electrons scattered out through a collimator edge the lateral movement of the electrons must also be considered When a plate collimator is ir

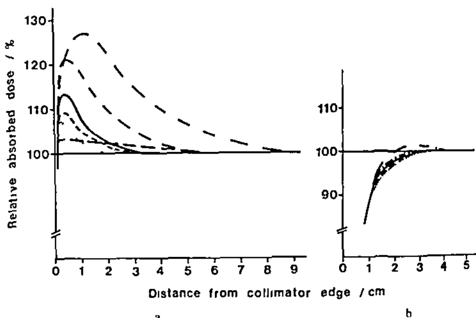


Fig 3 a) Relative absorbed dose in water due to electrons scattered out through a collimator edge These and consecutive curves show the ratio of absorbed dose in the field with and without collimator in place normalized to 100 per cent in the non collimated field The energy (E) = 10 5 MeV and the depth of measurement 1 mm Zero point of abscissa is the point where exactly half the detector is covered by the shadow of the collimator Relative absorbed dose at this point is close to 50 per cent b) Same as in (a) but depth of measurement 30 mm Comparison between (a) and (b) clearly demonstrates that almost all the collimator scattered electrons are stopped in the build up region (cf Fig 7) – – C H – – Al – Pb W – – Al+1 mm Pb

radiated a maximum distance exists from the edge of the collimator beyond which electrons no longer can be scattered laterally and escape at the edge This distance of maximum lateral displacement in a given material is also equal to the minimum beam radius necessary to obtain full broad beam conditions at the central axis of a circular beam incident on the material in question When the simple multiple scattering theory is used the distance of maximum lateral displacement may be approximated by the radius outside which the contribution to the fluence or the absorbed dose at maximum build up is less than one per cent This can be calculated when the lateral or radial electron fluence is known

In the Gaussian approximation the lateral displacement of the electrons in an electron beam can according to BRAHME (1975) be expressed by

$$\Phi(r\ z)=\frac{\exp\{r^2/\overline{r_z^2}\}}{\pi r^2} \qquad (9)$$

where r is the radial displacement from the z axis i e the central axis of the beam and $\overline{r_z^2}$ is the mean square radius of the radial distribution at the depth z (cf Fig 2) The radius R_1 outside which the contribution to the fluence on the central axis is less than one per cent is given by

$$\int_R^{\infty}\Phi(r\ z)\,2\pi r\,dr\leq 0\ 01 \qquad (10)$$

as $\Phi(r\ z)$ from eq (9) is normalized to one incident electron at $z=0$ From eq (10) R_1 is easily obtained

$$R_1=(\overline{r_z^2}\ln 100)^{1/2} \qquad (11)$$

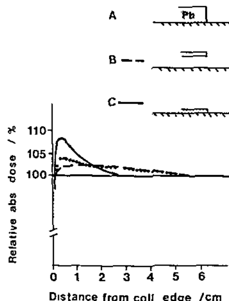
and the mean square radius $\overline{r^2}$ of a narrow beam incident at $z=0$ is according to BRAHME (19 given by

$$\overline{r^2}=\overline{\theta_0^2}z^2+Tz^3/3 \qquad (12)$$

Eqs (11) and (12) should be evaluated at the depth of dose maximum which may be approximated by the diffusion depth (BRAHME 1975) where the electrons have reached a state of full diffusion For most practical purposes the diffusion depth can be represented by the depth where the root mean square (rms) scattering angle is one radian (BETHE et coll 1938) This depth can be obtained from eq (4) and for a plane parallel beam it is ($\overline{\theta_0}=0$) given by

$$z_1=\left(\frac{T}{\varrho}+\frac{S_{t\ t}}{E_p\times\varrho}\right)^{-1}\varrho^{-1} \qquad (13)$$

Despite the somewhat arbitrary choice of one radian in the definition of z_1 and the approximate nature of



g 4 Relative absorbed dose in water due to electrons scattered it through a collimator edge of lead Geometric conditions in e 3 measurements are shown in the inserted figure A 25 mm ick lead collimator at surface of water phantom B 6 mm thick ad collimator 19 mm from the surface C 6 mm thick lead ollimator at surface Depth of measurement is 1 mm

q (4) at large depths the diffusion depth given by q (13) is an important transport parameter It learly shows that both the scattering and energy ss properties of the medium are involved By using qs (12) and (13) eq (11) reduces to

$$R_1 = \left(\frac{\frac{1}{3}\ln 100}{1+\frac{S_{t\,t}}{E_p T}}\right)^{1/} \times z_1 \approx z_1 \qquad (14)$$

ince the square root factor is fairly close to unity or most collimator materials and energies used in adiation therapy

As a first approximation z_1 may therefore be used s a measure of the maximum lateral excursion of he electrons In Table 1 the value of z_1 in mm is iven for some representative materials and energies f therapeutic interest It is quite clear that in high tomic number materials a much smaller region long the collimator edge will be available to scatter ut electrons at the edge

Absorbed dose due to edge electrons The total umber of electrons that will be scattered out hrough the collimator edge will thus depend on he product of two factors which partly compensate ach other First the number of laterally scattered lectrons in a narrow beam increases with atomic umber due to the increase in scattering power (cf eq 6) However this factor is normally counter balanced by the second factor which implies that a smaller area along the collimator edge is available to remove electrons laterally in high atomic number materials mainly due to their higher density (cf eq 14) The mean absorbed dose due to electrons scattered out through the collimator edge relative to the absorbed dose in the open field is thus proportional to the product of these two factors

$$D \propto N_x \times \left(\frac{T}{\varrho}+\frac{S_{t\,t}}{\varrho \times E_p}\right)^{-1} \varrho^{-1} \qquad (15)$$

which after inserting T (from ROSSI) and approximating S_{rad} by E_p/X_0 (the correct value at the high energy limit) appears as

$$D \propto \frac{E_p^{3/} X_0^{1/2} \varrho^{-1}}{E_p + E_p X_0 S_{col} + E^{\prime}} \qquad (16)$$

The absorbed dose due to electrons scattered out through a collimator edge is thus as expected inversely proportional to the density in addition to which it has only a weak dependence on energy and atomic number In the penultimate row of Table 1 relative values of D are given at 10 MeV normalized to water It is observed that D_c decreases by a factor of 10 on passing from water to lead Furthermore tungsten is almost three times better than brass

Method and Results

Beam parameters and dosimetry The experiments were carried out on a linear accelerator MEL 75 10 at one electron energy $(E_p)_0 = 10$ 5 MeV The energy was determined by range analysis according to NACP (1972) No scattering foil was used during the irradiations in order to reduce the angular spread of the electrons in the beam The vacuum window the monitor ion chamber the mirror for the light beam and the air volume between the phantom and accelerator have been the only scattering materials in the beam giving a field flatness of ±6 to 7 per cent within 5 cm from the central axis at depths from 1 to 30 mm The photon beam diaphragms of the machine have been fully opened (≃30 cm×30 cm) and the distance from the vacuum window to the phantom surface has been 100 cm The position of the effective electron source was 86 cm from the phantom as determined by ionization chamber measurements at two distances

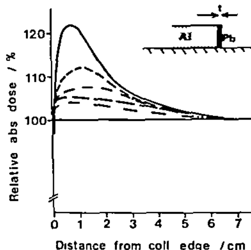


Fig 5 Relative absorbed dose in water due to electrons scattered out through a lead lined collimator edge Thickness t of lead given in mm and depth of measurement is 1 mm Thickness of collimator is 25 mm — t=0 0 10 - - 0 22 --0 44 - - 0 6 - x

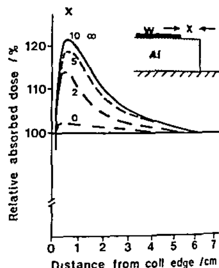


Fig 6 Relative absorbed dose in water due to electrons scattered out through a partly covered collimator Non-covered distance X in mm Thickness of aluminium collimator 25 mm and of tungsten plate 3 mm Depth of measurement is 1 mm

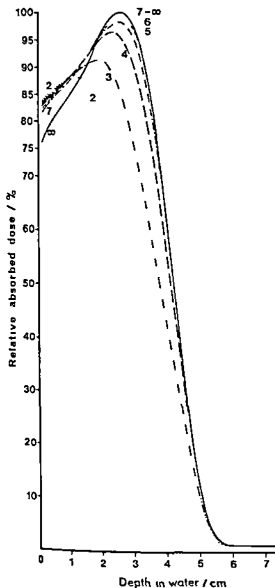
Measurements of the dose distribution were performed with an automatic isodose plotter (Scanditronix RFA 1) The detector was a 2 mm×2 mm semiconductor diode This detector was found to have no significant energy dependence at least between 3 8 and 25 MeV (HULTEN 1975) In the build up region the diode gives values which are too low compared with a liquid ion chamber (HULTÉN) The difference could be as much as 5 per cent and is greatest for beams approaching a monoenergetic and monodirectional beam (BRAHME & SVENSSON 1976) The difference is expected to be only 2 to 4 per cent in the present case (BRAHME & SVENSSON) due to the relatively high energy—and angular—spread in the beam mainly caused by the fairly thick monitor ion chamber

A slit of 1 4 mm width was made in a 3 mm thick tungsten collimator to check the geometric resolution of the detector A scan was made with the detector across the slit with a distance from the tungsten collimator to the semiconductor element of 2 mm This gave a FWHM of 2 5 mm at 10 5 MeV as would be expected from the size of the semiconductor element and the slit

A similar method was used to determine the size of the effective electron source Instead of the diode film was used as detector and the distance from the tungsten collimator to the film was 50 mm After correction for multiple air scattering behind the collimator eq (12) and the width of the slit as well as the size of the aperture of the density reader used the diameter or more exactly twice the root mean square radius of the effective electron source was determined to be 45 mm The angular spread of the beam was thus quite broad and the source size fairly wide though the scattering foil was removed

Collimator scattering The absorbed dose due to electrons scattered out through a collimator edge was measured at 1 and 30 mm depth in water for collimators made of polystyrene aluminium brass lead and tungsten (Fig 3) The collimators had a thickness of 5 to 10 per cent greater than the continuous slowing down approximation range r_0 (BERGER & SELTZER 1964) of the electrons in the material giving for polystyrene 55 mm aluminium 25 mm brass 8 0 mm and lead 5 7 mm at an energy of 10 5 MeV The mean range r_0 in tungsten is 3 2 mm but for practical reasons 3 0 mm was used The collimator was placed in direct contact with the water phantom with the edge of the collimator on the central axis of the electron beam The dose profile was measured perpendicular to the direction of the collimator edge and the measurement at each point was related to measurement in the uncollimated open field The effect of a laminated collimator was also evaluated using an aluminium collimator covered with a lead layer at the edge (Fig 3 a)

The relative values of the mean absorbed dose contribution from collimator scattered electrons normalized to the theoretical value 0 91 for polystyrene (C_8H_8) appear in Table 1 The mean absorbed dose has been estimated by integrating the



ig 7 Central axis depth dose curves for beams with diameters om ? cm in steps of 1 cm up to 7 cm and ∞ (30 cm×30 cm y photon collimators) Curves for 7 cm and ∞ are identical eyond a depth of 1 8 cm Within this depth a clear difference t relative dose between open and collimated beam due to con ibution from electrons scattered out through collimator edge

rea above the 100 per cent level in Fig 3a The greement with the theoretical values from eq (16) is good The small discrepancies may be due to incertainties in the dosimetric techniques and to approximations in the theoretical evaluation

A series of measurements were performed (Fig 1) in order to separate the dose contribution from lectrons reflected at the edge (Fig 1 ray No 2) and those scattered out through the edge (Fig 1 ray No 4) In the first measurement a lead col limator of 25 mm thickness was placed at the surface of the water phantom (A in Fig 4) In a second measurement a lead collimator of 6 mm thickness was positioned 25−6=19 mm from the water phantom (B in Fig 4) The difference between the two measurements is due to electrons reflected at the surface of the collimator edge from a lead height of 19 mm The contribution to the absorbed dose from reflected electrons is thus for most purposes negligible in comparison with the contribution from electrons scattered out through the collimator edge It can also be seen in Fig 4 that the reflexion angle is small as expected (MANDOUR & HARDER 1975) when the collimator edge is well aligned with the electron source The curve for a 6 mm lead collimator placed at the surface of the water phantom is illustrated in C in Fig 4 This clearly demonstrates the difference in dose distribution caused by different geometries In cases B and C the same number of electrons is scattered out and their angular distribution is the same

Due to the greater distance from the surface in case B the electrons are distributed over a larger area The geometric effect mentioned explains part of the favorable effect obtained when a high atomic number lining is added to a low atomic number collimator (Fig 3a) Alternatively it is possible to have a few cm of clearance between the last collimator and the surface to avoid the hot spots near the edge of the collimator (Fig 3) However the laminated collimator is the best choice at high energies when the bremsstrahlung production must be minimized The effect of different lead thicknesses on an aluminium collimator was measured and the results are given in Fig 5 It may be concluded that a thickness of about 0 $5_{e,1}$~0 5 mm is for practical purposes sufficient to remove most of the scattered electrons from the aluminium collimator The use of tungsten instead of lead would be even more effective (Table 1) and a thickness of 1 3 mm W (0 $5_{e,1}$) would be enough for an energy of 40 MeV

The contributions of different lateral layers (X Fig 6) in an aluminium collimator to the number of electrons scattered out of the edge were evaluated in a separate measurement illustrated in Fig 6 The most laterally displaced electrons give the smallest contribution to the absorbed dose in the beam About 90 per cent of the electrons scattered out through the edge originate within 0 $5_{e,1}$ from the edge of the collimator

Field size dependence An aluminium collimator covered with 1 mm of lead at the edge gives an

additional dose at the edge of the field from collimator scattered electrons of only a few per cent in a broad beam (Fig 3a) This type of collimator has therefore been used to measure the field size dependence of the depth absorbed dose curve

The diameter of the circular diaphragms in the collimator plates ranged from 20 mm up to 70 mm in steps of 10 mm the thickness of the collimators was 25 mm Again the collimator was placed in direct contact with the surface of the water phantom concentric with the central axis of the beam The relative depth dose curve was measured on the central axis of the collimated beam

The results of the measurements appear in Fig 7 The curves are normalized to 100 per cent at dose maximum in the opened beam The precision in the measured curves is about one per cent

A number of important conclusions can immediately be drawn from these results The mean energy of the electrons scattered out of the edge is only about 40 per cent of the mean energy of the incident electrons eq (8) This means that only the build up region is influenced by the collimator scattered electrons this is evident in Figs 7 and 3b The dose at one mm depth is increased from 76 per cent to 82 per cent when the beam is collimated from the open field to 7 cm diameter For depths of more than 18 mm the two curves are almost identical since most of the collimator scattered electrons are lost The use of a low density collimator may therefore be a valid method for increasing the surface dose for the treatment of small superficial lesions

The general shape of the narrow beam curves agree well with the recent Monte Carlo results of BERGER & SELTZER (1978) It is evident that the practical range no longer is suitable for energy determination when the beam cross section is much smaller than z_i since the usual energy range relation is violated

The absorbed dose at the depth of dose maximum in a collimated beam is compared with that of an open beam in Table 2 It can be seen that the absorbed dose has decreased by one per cent at a field cross section of 6 cm This is in fair agreement with the approximate value $2z_i$ as deduced in eq (14) The value of $2z_i$ is 7 cm for water at 10 MeV

The value of z_i in Table 1 can thus be used as the minimum radius for obtaining broad beam conditions in a uniform beam

Table 2

Field size dependence of the maximum absorbed dose at 10.5 MeV

Field size (cm)	Relative absorbed dose (per cent)
Open field (30×30)	100
Ø 7	100
Ø 6	99
Ø 5	98
Ø 4	96
Ø 3	91
Ø 2	88

Conclusion

Electrons which enter a collimator on the s[illegible] side and are scattered out through the collim[illegible] edge are often the most important form of be[illegible] contamination in electron beams They can a[illegible] significant doses to the build up region when lo[illegible] density collimators are used This effect is mini[illegible] for high density collimators separated from t[illegible] patient by a few cm or which is even better fr[illegible] the photon contamination point of view a lo[illegible] atomic number collimator with a high density la[illegible] having a thickness equal to half the diffusion de[illegible] in the material in question

It is demonstrated that the diffusion depth is t[illegible] relevant parameter for comparison with the fi[illegible] size to evaluate whether broad beam dose distrib[illegible] tions are obtained on the central axis of the bea[illegible] Furthermore it may be concluded that the use [illegible] the collimator to improve the uniformity of an [illegible] sufficiently flattened electron beam is a very p[illegible] method since it has a negligible effect from the d[illegible] maximum down to the therapeutic range Howev[illegible] on such accelerators it is of little value remov[illegible] the collimator scattered electrons without si[illegible] taneously improving the beam flattening since th[illegible] electrons do contribute somewhat to the uniform[illegible] in the superficial layers particularly when the co[illegible] limator is in direct contact with the skin

SUMMARY

The collimation of high energy electron beams for rad[illegible] tion therapy is treated with special attention on t[illegible] contamination of the beam by electrons which ide[illegible] should be stopped but instead are scattered back [illegible] the beam through the edge of the collima or It is [illegible]

trated that the mean energy of these electrons is only per cent of the mean energy in the beam and that smallest electron contamination is obtained if the erial closest to the beam is of high density

ACKNOWLEDGEMENT

lany valuable suggestions of Lars Eric Larsson Hans nsson and Rune Walstam are gratefully acknowledged t of this investigation was supported by grants from g Gustaf V Jubilee Fund

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)M THE POLYTECHNIC OF CENTRAL LONDON LONDON WIM 8JS ENGLAND AND THE DEPARTMENT OF RADIATION PHYSICS KAROLINSKA INSTITUTET S 10401 STOCKHOLM SWEDEN

MICRODOSIMETRY

II Use of secondary electron emission to simulate two target models

T E BURLIN and B J FORSBERG

S condary electron emission has been suggested a phenomenon offering considerable potential for crodosimetric analyses (BURLIN 1974) Previous an exploration of secondary electron emission as alternative technique for microdosimetry was re rted by FORSBERG & BURLIN (1980) who calcu ted lineal energy distributions from derived imber distributions of secondary electrons (<50 /) from the exit and entry sides of thin tissue uivalent foils bombarded with keV electrons iose distributions can be considered as single lume distributions i e the number of secondary ectrons emitted per primary particle was cal ilated only on one of the sides

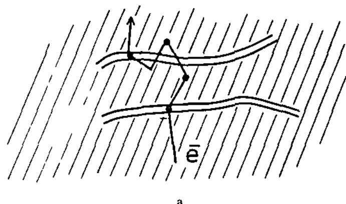
Furthermore measurements of the variance of the condary electron current have been performed ORSBERG 1978) and the derived dose mean value lineal energy indicates that the secondary electron nission should be a potential technique for mic dosimetry

In the present report coincident single event dis ibutions from the two surfaces of thin foils of car on and lithium fluoride are calculated i e the umber of secondary electron pairs (n_i n) where n the number of secondary electrons leaving the icident escape zone and n the number leaving the xit escape zone of the foil simultaneously These alculations were performed to show the potential of possible experimental technique which simulates bject sizes of a few nm separated by up to several hundreds of nm and could thus be used to investi gate the energy deposition in two related volumes

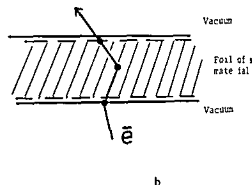
Background Several authors have suggested that some biologic effects may result from energy deposi tion in two different targets (NEARY 1965 ROSSI 1968 ALPER 1970) NEARY suggested that the targets were in the nanometer region and separated by a distance not exceeding 0 2 μm KELLERER & ROSSI (1971) concluded that for cells of higher or ganism the targets were of nanometer dimensions separated some micrometer An experimental inves tigation with proportional counter measurements to the two target theory has been reported previously (BURLIN et coll 1972 BENSTOCK et coll 1974 1976) One of the limitations with this technique is the difficulty to simulate target diameters less than 0 5 μm two orders of magnitude greater than the size of such a biologic target In secondary electron emission the relevant volume is a very thin layer the shape would thus be equivalent to a thin membrane which has been proposed by ALPER as a sensitive structure in her two target theory ALPER suggested that damage from radiation in non nucleic acid sites can be identified as lesions in membranes A cell death could then occur if DNA or an enzyme was attached to a particular site on the membrane and an interaction took place between the two volumes

The cell membranes vary in thickness due to if

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a



b

Fig 1 a) Two biologic targets which can be simulated by the experimental arrangement shown in (b) b) Thin foil in vacuum irradiated by electrons The dotted lines indicate the esca zones at the incident and exit side

they consist of a single layer then approximately 10 nm thick or a multi layer If several very thin layers exist in the membrane it may be important not only to analyse the stochastic behaviour of energy de position in one layer but also in two or more as sociated layers The shape and thickness of those layers are similar to the escape zones in secondary electron emission In the present report an alterna tive technique to twin proportional counter meas urements based on the secondary electron emission technique is discussed and some calculations are made to examine the potential of the technique

Secondary electron emission as an alternative technique for microdosimetry has previously been treated in detail for a single target by FORSBERG & BURLIN

When a medium is irradiated by ionizing radiation a degradation spectrum is established within the medium When this electron spectrum passes through the interface between the material and a vacuum some of the electrons will penetrate the potential barrier and escape from the surface with an energy of 0 to 50 eV These only will be referred to as secondary electrons in the following The cut off at 50 eV is of course arbitrary but has been widely accepted in the literature on secondary electron emission The secondary electron spectrum has a marked peak at 1 to 3 eV outside the medium

The secondary electrons have a very limited range in a solid due to the interaction with the electrons and phonons so that secondary electrons emitted originate from a thin surface layer This layer from which secondary electrons are able to escape is known as the escape zone Its thickness is charac teristic of the particular material but is independent of the energy of the primary radiation The maximum depth of the escape zone is about 10 nm for metal and about 5 times this value for insul tors (DJATLOWITSKAJA 1948 NAKHODKIN & ROMANOWSKY 1958 BRONSHTEIN & SEGAL 1960) Thus secondary electron emission reflects ener depositions within volumes of linear dimensions of approximately 10 nm which is much smaller tha can be simulated by the conventional proport counter technique which is limited to approxima ly 0 5 μm or more

If the low energy secondary electrons are re corded at the incident and exit surface related ene gy depositions in two nm thick volumes separated distance approximately equal to the foil thickness can be determined (Fig 1) Fig 1 a shows two pa allel chromatin targets a few nanometer thick separated by an interaction distance of several te of nanometers The probability of one inelastic in teraction in one of the targets could be simulated b analysing the energy deposition at the incident exit escape zone (Fig 1 b) In the same way th probability of at least one inelastic interaction o curring simultaneously in both biologic targets c be simulated by determining the probability that each of the two escape zones an inelastic interacti occurs for the same primary particle All the calc tions in the present report are based upon the situ tion illustrated in Fig 1 b

In traditional microdosimetry mu h effort h been put into the use of wall less detectors th reason for this being the so called wall effects (KE LERER 1971) These effects are caused by the diffe ent density of the gas and of the walls of the propo tional counter In secondary electron emission a hence in the present calculations opposite eff will occur electrons are coming from vacuum i

d foil and after interacting in that foil the elec ns sometimes will be emitted out to the vacuum in Of three wall effects mentioned by KEL ER the analogue to the re entry effect will be the st important if the backscattering from polarising trodes or other material is ignored (Fig 1) In a nogeneous situation (Fig 1 a) an electron can be kscattered and re enter the sensitive volume and se interactions there The re entered electron l be missed with the secondary electron emission hnique unless a backscattering material is intro ed The situation for which the calculations were de will thus underestimate the energy deposited other two effects the V effect and the δ ray ct will be small due to the limited range of the ay particles for keV electrons used in the calcula ns

imulated object size and target distance The of the simulated object can usually be derived follows The depth of the escape zone i e the ximum depth from which the most energetic (50) secondary electrons are able to escape is taken m the literature The probability of escape de ases with depth and is described by an exponen function The mean escape depth (or the charac istic escape length) is usually taken as one third of escape zone Since in microdosimetry the mean ord length in unit density material is of more in est than the actual dimensions the mean escape oth is multiplied with the density of the foil and a nstant (=2) This constant was taken from BIRK FF et coll (1970) who derived the mean chord gth for infinite thin slabs irradiated under iso- pic conditions This was used though in the pres investigation the primary electrons were di ted perpendicular to the foil This means that the ean chord length given will be an overestimation t it is accurate enough for the present purpose

The mean chord length at the incident side will vays be smaller than at the exit side The reason this is that the primary electrons reach the exit rface layer at different angles and a more iso- pic situation occurs This will tend to reduce the ergy deposited per event at the incident side com red with the exit side which is also observed in calculated distributions

The thickness of the escape zones for LiF and C is taken to be 24 and 4 nm respectively A 100 nm ck LiF foil will thus simulate two unit density lumes with mean chord lengths 2×ρ× (descape ne)/3=42 nm separated by a unit density layer of approximately ρ×d=250 nm Similarly a graphite foil could simulate two unit density volumes with mean chord lengths of 6 nm using a density for carbon of 2 25 g/cm³

Calculations The calculations were based on a Monte Carlo program by McDONALD et coll (1971 1973) and were described together with necessary input parameters to the program by FORSBERG & BURLIN

If the calculated probability P(n) that n secon dary electrons will emerge per primary electron is multiplied by the average energy expended in the escape zone per secondary electron emitted the probability distribution of lineal energy is obtained

The two-target situation postulated by several au thors referred to in the introduction may also be simulated by extending these calculations The escape zones at the incident and exit sides of a thin foil simulate the two small volumes (nm) and the thickness of the foil (μm) simulates the interaction distance The number of secondary electrons re leased by a single primary electron from both the incident and exit surfaces of the foil was calculated Each primary electron was incident perpendicular on thin foils of carbon or lithium fluoride with actual thicknesses of maximum 0 1 μm and 0 4 μm i e approximately 0 23 μm and 1 μm respectively in unit density material During its passage through the foil the electron will lose energy in discrete steps and produce low energy electrons Sometimes sec ondary electrons will emerge from both the incident surface (n_i) and the exit surface (n_e) resulting from energy deposition by the same primary particle (event) giving rise to a coincident event Registration of the number of secondary electrons in the form (n_i n_e) including the most common situation (0 0) (n 0) and (0 n) was undertaken through an extension of the Monte Carlo program

Results and Discussion

It was intended to explore the potential of secon dary electron emission as a technique for mic rodosimetry and especially its possible use to obtain information about the energy deposition in two re lated targets Only a few examples will be given

Figs 2 and 3 show P(n)-distributions for emis sion of secondary electrons at both the incident and exit surfaces The information obtained may be pre sented in various ways It is most probable that a fast electron will pass through the incident escape

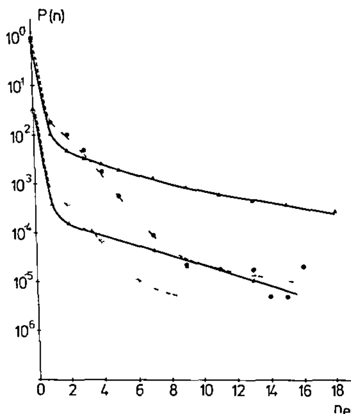


Fig 2 Probability distributions P(n) at the incident and exit surface of a 0 1 μm thick carbon foil irradiated by 6 keV electrons Points show calculated results ▲ Number of secondary electrons emitted from the exit surface when no secondary electrons are emitted from the incident surface $n_i=0$ ● Number of secondary electrons emitted from the incident surface when no electrons are emitted from the exit surface $n_e=0$ △ Number of secondary electrons emitted from the exit surface when at least one electron is emitted from the incident surface $n_i>0$ O Number of secondary electrons emitted from the incident surface when at least one electron is emitted from the exit surface $n_e>0$

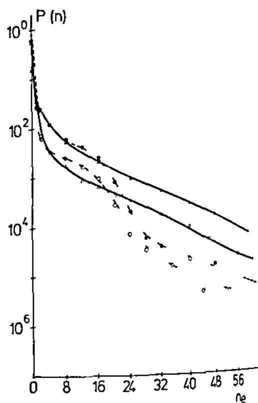
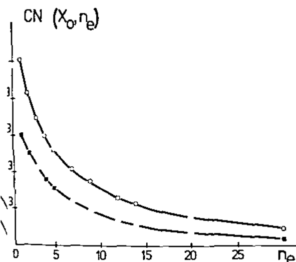


Fig 3 Probability distributions P(n) at the incident and ... surface of a 0 1 μm thick lithium fluoride foil irradiated by 6 ... electrons Points show calculated results Symbols as in Fig ...

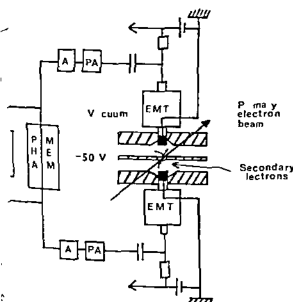
zone without the emission of a secondary electron the probability of releasing 0 1 2 etc secondary electrons as it passes through the exit escape zone appears in Fig 2 Such an event would not lead to biologic action involving the two targets unless energy was deposited in the other target by another fast electron before repair processes had intervened Thus a two target model is simulated when at least one secondary electron is also emitted from the incident escape zone This is also shown in Fig 2 as the probability that 0 1 2 etc secondary electrons are emitted from the exit escape zone when at least one electron is emitted from the incident escape zone The same figure also shows distributions giving the probability of escape of a secondary electron from the incident escape zone when zero (single volume) and when at least one secondary electron (two targets) are emitted from the exit escape zone It is evident that the shape of the probability curves is not greatly changed by the coincident requirement

but the magnitude of probability of an event wh... can lead to biologic action decreases by about tw... ty times As shown in Fig 2 there are two sets ... curves with different shapes which depends ... whether the secondary electrons (on the absci... are emitted from the incident or exit surface L... The mean chord length for primary electrons in ... incident volume will be smaller than in the exit ... ume and hence the probability of la ge number... secondary electrons being emitted from the incid... side is low compared with the probability on the e... side The data in Fig 2 relate to a 100 nm th... carbon foil bombarded with 6 keV electrons T... thickness of the escape zone for carbon is appr... mately 4 nm This would simulate in tissue eq... lent material two targets with mean chord length... about 6 nm separated by some 0 23 μm

Similar calculations were undertaken for 1 ... fluoride foils bombarded with 6 keV electrons (F... 3) The escape zone thickness for LiF is a...



4 Number of coincident events for 2×10^6 primary electrons $(X_0\ n_e)$ resulting in emission of at least n_e secondary electrons the exit surface of two lithium fluoride foils irradiated by 6 electrons ■ Thickness=0 05 μm O Thickness=0 1 μm



5 Experimental arrangement to determine the probability distributions P(n)

itely 42 nm Therefore this simulates in unit den y material the situation where two targets with an chord lengths of approximately 42 nm are parated by about 0 25 μm The general shape of probability distributions both in the case where secondary electron is emitted from the incident cape zone when a fast electron passes through d also in the incident escape zone are very similar the results for carbon

Figs 2 and 3 together illustrate the effect of differ t mean chord lengths The ratio between the single and coincident interactions is markedly smaller for LiF than for carbon which is expected because of the longer mean chord length of the primary electrons in the escape zone of LiF

In Fig 4 the number per 2×10^6 primary electrons of coincident events when at least n_e secondary electrons are emitted at the exit side is shown at two LiF foil thicknesses i e at different target separations Thus $CN(x_0\ n_e)=\Sigma(n\ n_e)$ where $n\neq0$ and x_0 is the actual foil thickness For $n=1$ $(n\ n_e)\neq0$ is approximately twice as probable for the thicker foil than for the thinner foil and this probability ratio increases as n_e increases

Similar calculations for carbon and LiF of approximately the same foil thickness and primary electron energy (6 keV) show that it is about 30 times more usual to get any $(n\ n_e)\neq0$ for LiF than for carbon

The data presented are the essential information to provide lineal energy distributions While the calculations have only been undertaken for electrons such calculations can in principle be extended to *other ionizing radiations However that is out of* scope of the present report which is aimed at illustrating that an alternative experimental technique exists by which microdosimetric data can be obtained This technique simulates target sizes in the nanometer region which are not readily simulated by techniques based on gas ionization

It should be of interest to check the calculations through measurements A possible arrangement is schematically shown in Fig 5 Two electron multiplier tubes are used for the detection of the low energy electrons but other systems could be used as well In the arrangement illustrated in Fig 5a thin foil of conductive material is irradiated with gamma rays These electrons will interact in the foil and secondary electrons will be emitted at the incident and exit side If the foil is placed at a low negative retarding potential the secondary electrons will move towards electron multipliers in which they will be multiplied 10^8 times The tubes are then connected to the charge sensitive preamplifier and so on Coincident pulses can be stored via a pulse height analyser It should be possible to place the foil between two slabs of the same material as the foil and thus avoid perturbing significantly the charged particle spectrum It may be neccessary to include some grids to collect the low energy electrons from the slabs themselves All secondary electron emission work is critically dependent on the

nature of the surface Consequently careful attention would have to be given to calibration techniques as well as to the condition of the surface

SUMMARY

A potential technique to analyse related energy depositions in two adjacent tissue equivalent volumes is described The technique is based on secondary electron emission from the two surfaces of a μm thick foil The foil should thus simulate two nm thick slabs separated a distance equal to the thickness of the foil Calculations of the coincident probability i e the probability that any secondary electrons are emitted from the front surface when at least one secondary electron is emitted from the exit surface is presented to show the potential of the technique An experimental arrangement is also discussed

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CHROMOSOME COUNTS OF ^{90}Sr-INDUCED OSTEOSARCOMAS IN MICE

III Variation of the chromosome counts of in vivo transplanted tumours, in vitro cultures and retransplanted cultured cells

H BERGMAN

As it is still technically difficult to obtain suc ssful chromosome specimens directly from solid mours an alternative method may be the use of a ng term cultivation procedure However this type technique may not reflect the true internal milieu a tumour but instead create a completely new ivironment for the growing tumour cells This in udes impairment by factors related to the remote id local defence mechanisms and loss of parame rs due to the microcirculation and oxygen tension the intact tumour etc Such factors may well fect different cell types and perhaps also the iromosome pattern as a histologically well fined type of tumour may be and probably is a ixture of aetiologically different subclasses each ne possibly with its own special chromosome ab- ormalities Furthermore the question may arise hether it is only tumour cells or stroma cells or a ixture of both that grow For this reason the pri iary purpose of the present investigation was to nalyse the conformity in chromosome patterns be ween cultured cells from ^{90}Sr induced and parallel in vivo transplanted tumours Another intention as to examine the consequence of retransplanting ultured cells to hosts with an apparently normal nmunologic response ^{90}Sr induced osteosarcomas vere selected as an experimental model as these umours had previously been examined for numer us characteristics (NILSSON 1962 1966 1969 1971 NILSSON & RÖNNBÄCK 1973) including the numeri al chromosome progression of serially transplanted tumours (Part I BERGMAN & NILSSON 1980 Part II BERGMAN 1980)

Material and Methods

Transplantation procedure Only inbred about 60-day old CBA mice were used The primary os teosarcoma was obtained from a mouse which had received an injection of 29 6 kBq $^{90}Sr(NO_3)$ /g body weight The tumour was localized by radiography 288 days after the injection From this primary tumour the numerical chromosome progression of 55 serially transplanted generations was analysed and presented as transfer series A in a previous report (Part I) The present investigation started with transfer generation A8 Tumours from this gen eration were thus continuously used for in vivo transplantation (Fig 1) but also for a parallel long term cultivation designated cultures 8 1 to 8 3 The cultures corresponded in time to the in vivo generations A9 to A11 Cells from culture 8 1 were then used for retransplantation to mice (trans fer generation a12) and from the new outgrown tumours serial in vivo transplantation was per formed (transfer series a)

Tissue culture methods The tumour cultures were obtained by mincing pieces of tumour in Minimum Essential Medium (MEM) with Earle s salts Tissue fragments were placed between cov

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erslips in a Leighton tube containing tissue culture medium 80 per cent MEM and 20 per cent foetal bovine serum plus 100 IU penicillin streptomycin per ml to 100 ml MEM was added 1 ml L glutamine After incubation at 38 °C for 48 hours the cells were rinsed with Hank s BSS removed from the coverslips with trypsin and transferred to culture bottles Culture solution was added and after 3 to 5 days growing cell patches were observed The outgrown cells were then transferred to another bottle after about one week Altogether 8 passages were obtained When the tissue cultures were harvested some of the cells were pretreated with Colcemid to a final concentration of 10^{-7} M These cells used for chromosome analysis were incubated in hypotonic solution (sodium citrate 1 %) and fixed in 1 part glacial acetic acid plus 3 parts ethyl alcohol The fixation procedure was repeated three times The remainder of the cultured cells from culture 8 1 was used for retransplantation These cells were subjectively diluted with an isotonic sodium chloride solution Ten mice were then injected subcutaneously with 0 2 ml cell suspension From the new outgrown tumours (transfer generation a12) serial in vivo transplantation was performed

Chromosome analysis The method used for preparation of chromosome specimens has been described previously (Part I) Only apparently well spread and unbroken metaphases were examined Altogether 5 tumours per transfer generation were used In successful preparations at least 25 cells per tumour were analysed However in poor specimens it sometimes was necessary to use a reduced number and some transfer generations yielded no well spread metaphase Due to the rather few chromosome counts per tumour and except for the tumours of transfer generation A8 and the cultures 8 1 to 8 3 only the percentile chromosome distribution per transfer generation is presented

Histologic examination Sections of the tumours were fixed in Stieve s solution embedded and stained according to the van Gieson method or with haematoxylin eosin The definition of tumour classification had been presented previously (Part I)

Results

Recording of chromosome abnormalities was limited to numerical variation and metacentric chromosomes Thus numerous accidental chromosome aberrations were not recorded

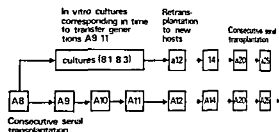


Fig 1 Diagram of in vivo transplantations (A8–A45 and a1 … and in vitro cultures 8 1–8 3

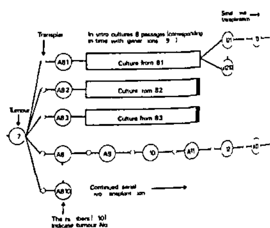


Fig 2 Diagram illustrating in vivo transplantations from transf generations 7 to 13 (each generation consisting of 10 tran pl and the parallel in vitro cultures plus retransplantation of cultur cells

In Fig 1 a diagram of the transplantations is presented and in Fig 2 a diagram of the transplantations but with special reference to the in vitro cultivatio and the retransplantation procedure Figs 3 to 5 d play cells with different chromosome numbers Dif ferent characteristics of the transfer generations a the in vitro cultures are recorded in Tables 1 and respectively and the percentile chromosome dis tribution per generation and for the cultures in Table 3 The individual chromosome pattern of the 4 tumours (tumours 8 1–8 3) used for separate in vit ro cultivation the chromosome count of the cultures (cultures 8 1–8 3) and the variation in chromosome distribution obtained when cells from culture 8 1 were retransplanted to mice to induce new tumours (transfer generation a12) are presented in Tables 4 and 5

Chromosome distribution The initial tumours used were derived from the 8th transfer generation These tumours displayed a predominating number of cells within the 35 to 39 region and with peaks at 37 (43 2 %) and at 35 (20 5 %) The succeeding tra fer generations (A9 and A10) had apart from a ce



Fig 3



Fig 4



Fig 5

Fig 3 A 38-chromosome cell from a tumour of transfer generation A8

Fig 4 A 59-chromosome cell from a tumour of transfer generation a_0

Fig 5 A 69-chromosome cell from a tumour of transfer generation A1?

ain percentile variation a similar chromosome distribution

Unfortunately no successful preparations were obtained from the 11th generation The cell cultures 1 to 8 3 were run parallel in time with transfer generations A9 to A11 When comparing the chromosome pattern of the cultured cells with the in vivo generations a quite different distribution is revealed Thus no cells with a lower chromosome number than 52 were found in the cultures but instead a predominating number of cells within the 56 to 64 region and with peaks at 60 (21 3%) When cultured cells were retransplanted to mice (transfer generation a12) tumours with a modified chromosome pattern appeared The major changes consisted primarily of a predominating number of 40-chromosome cells (51 7%) and a more irregular distribution within the triploid region In contrast to this generation the parallel A12 generation displayed a majority of cells within the 35 to 42 region

Table 1

Different characteristics of the in vivo generations examined

Transfer generation	No of tumours examined	No of cells counted	Mean growth period (days)	Mean size (cm³)	Mean growth per day (cm³)	Predominating chromosome No	Per cent	Percentage of cells with metacentrics
A8	3	132	24 3	0 73	0 030	37	43 2	9 8
A9	4	101	19 5	0 94	0 048	37	59 4	5 9
A10	3	75	20 3	1 07	0 053	37	48 0	10 0
A12	6	150	16 5	0 79	0 048	37	31 3	5 3
A14	4	95	20 5	0 88	0 043	37	48 4	0
A20	3	75	15 3	0 87	0 057	40	84 0	0
A25	3	75	14 7	0 95	0 065	40	90 7	0
A45	3	75	15 7	0 84	0 054	40	41 3	1 3
a1	4	89	22 0	1 01	0 046	40	51 7	2 2
a14	4	92	19 0	0 98	0 052	40	46 7	0
a20	7	174	16 6	1 25	0 075	60	28 7	2 3
a25	4	100	14 0	0 87	0 062	60	16 0	0

and with peaks at 37 (31 5%) and now also at 40 (24 7%) The next a generation from which successful chromosome preparations were obtained was a14 The percentage of 40-chromosome cells was now found to be 46 7 per cent and within the triploid region numerous cells within the 62 to 67 region but without distinct peaks From generation A14 a preponderant number of 37 chromosome cells was still recorded while the 40 chromosome cells had decreased (5 3%) The next comparison was between generation A20 and a20 The A series was here characterized by a high frequency of 40-chromosome cells (84 0%) while the a series had only 10 3 per cent and within the triploid region frequently appearing cells within the 56 to 63 region and with a peak at 60 (28 7%) The final comparison was between generations A25 and a25 Concerning the A generation an even more marked dominance of 40-chromosome cells (90 7%) was recorded

Table 2

Data of the in vitro cultures (8 1–8 3)

No of passages	8
Growth period days	60
Corresponding transfer generation	A9–11
No of tumours examined	3
No of cells counted	150
Predominating chromosome No	60
Per cent	21 3
Percentage of cells with metacentrics	0

while generation a25 showed distinct similarit with the preceding a generation Sporadic analyse of the A series showed that the hitherto last ex amined transfer generations (43–55) were chara terized by numerous 40-chromosome cells (41 71 0%) but also by cells within the 52 to 62 region

Metacentric configurations The percentage cells with one or more metacentrics ranged from 0 10 (Tables 1 2) The most metacentrics were foun in the earlier transfer generations (A8–A12) of th continuously in vivo transplanted A series while fo instance no metacentrics were found in the cultur (8 1–8 3)

Histologic characterization The primary os sarcoma A was characterized as an osteosarco of predominantly fibroblastic type and with a sli osteoid formation (Part I) *Samples* obtained fr tumours of transfer generations A8 to A12 we classified as highly differentiated fibroblastic o teosarcomas with a solid growth and with polym phic cells In the succeeding transfer generations th *A tumours were transformed* into more and mo anaplastic entities while this transformation was n as evident in the a series Concerning the cell cul tures (8 1–8 3) no histologic classification was pe formed However of special interest was to rec that the tumours of transfer generation a12 i e tumours which grow out after retransplantation o cultured cells to mice were classified as highly d ferentiated osteosarcomas with trabecular and growth

Table 3

The percentile chromosome distribution of the transfer generations and the in vitro cultures examined

nsfer era	No of tumours examined	No of cells counted	32	34	35	36	37	38	39	40	41	42	43	44	45	48	50	51	52
omosome number			32	34	35	36	37	38	39	40	41	47	43	44	45	48	50	51	52
	3	132	0 8	1 5	?0 5	8 3	43 ?	7 6	6 8	0 8	0 8						0 8		
	4	101			2 0	12 9	59 4	11 9	?0	3 9					1 0				
	3	75		?7		8 0	48 0	13 3	1 3	17 3	1 3		1 3						
	6	150		0 7	2 0	6 7	31 3	7 3	1 3	24 7	6 0	2 0	0 7						
	4	95			4 2	10 5	48 4	9.5	1 1	5 3									
	3	75					1 3	2 7	5 3	84 0	6 7								
	3	75					1 3		2 7	90 7	?7								
	3	75								41 3		1 3							
tures	3	150																	1 3
	4	89							2 ?	51 7						1 1		1 1	
	4	9?							?2	46 7	2	3 3		??					
	7	174							0 6	10 3									
	4	100								13 0									

omosome number			53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69
	3	13?																	
	4	101										1 0							
)	3	75																	
	6	150													0 7		1 3	0 7	1 3
	4	95																	
)	3	75																	
5	3	75								1 3									
5	3	75		1 3	?7			5 3	5 3	?8 0	10 7	4 0							
ltures	3	150		0 7		5 3	7 3	10 7	10 0	21 3	14 0	13 3	4 7	3 3	1 3	2 7	1 3		
	4	89	1 1	3 4	5 6	3 4	3 4	4 5	1 1		1 1	1 1		3 4	5 6	4 5	4 5		
	4	9							1 1		1 1	5 4	2 2	8 7	5 4	8 7	6 5	1 1	
	7	174			1 1	3 4	8 0	19 0	14 9	?8 7	6 9	2 9	2 3			0 6			
	4	100	5 0		4 0	7 0	13 0	7 0	9 0	16 0	1? 0	6 0	5 0	1 0	1 0				

romosome number			70	71	72	73	74	75	76	77	80	86	88	90	92	94	123	136
	3	132	0 8		1 5	0 8	4 5	1 5										
	4	101				3 0	2 0		1 0									
0	3	75			2 7		1 3		1 3		1 3							
	6	150	2 0	?0	0 7	2 0	2 7	0 7	0 7	1 3				0 7				0 7
4	4	95		1 1	5 3	6 3	3 2	1 1				1 1	1 1		1 1	1 1		
0	3	75																
.5	3	75									1 3							
l5	3	75																
ltures	3	150			1 3	0 7				0 7								
2	4	89															1 1	
4	4	92	1 1	1 1							1 1							
0	7	174			0 6					0 6								
5	4	100		1 0														

Cell cultures (8 1–8 3) corresponding in time to transfer generations A9–11

Conclusions

The main purpose was to attain an idea whether a hanged selection pressure obtained by what may e designated an immunologic defenceless in vitro environment affects the chromosome pattern in comparison with the parallelly and serially trans planted tumours examined by a direct method and to analyse the result of retransplanting cultured cells

Table 4

The chromosome distribution of the three tumours (A8 1–A8 3) used for separate in vitro cultivations plus the chromosome distr[illegible] of the cultures (culture 8 1–8 3)

Tumour or culture No	No of cells counted	Chromosome number																	
		32/34	35	36	37	38	39	40	41	42/51	52/55	56	57	58	59	60	61	6[illegible]	6[illegible]	[illegible]
A8 1	48	1		2	29	5	2		1	1										
Culture 8 1	100											4	3	11	11	8	16	15		5
A8	59	[illegible]	27	6	13	2	4	1												
Culture 8	25										2	2	5	2	1	1	3	3	4	
A8 3	25			3	15	3	3													
Culture 8 3	25										1	2	3	3	3	3	[illegible]	[illegible]	1	

Table 5

Variation in chromosome distribution obtained after in vitro cultivation (culture 8 1) and retransplantation of cultured cells (tr[illegible] generation a12) from tumour 8 1 For comparison the chromosome pattern of the in vivo transfer generations A12 and A45 is prese[illegible]

Tumour culture or transfer generation No	No of cells counted	Chromosome number																		
		34	35	36	37	38	39	40	41	42	43	44/49	50/53	54	55	56	57	58	[illegible]	[illegible]
Tumour A8 1	48	1		2	29	5	2		1				1							
Culture 8 1	100															4	3	11	11	3
Generation a12	89						2	46				1	2	3	5	[illegible]	3	4	1	
Generation A12	150	1	3	10	47	11	2	37	9	3	1									
Generation A45	75							31		1				1	2			4	4	1

to animals with a normal immunologic response The main findings can be summarized as follows

(1) From a ^{90}Sr induced osteosarcoma a transfer generation A8 was established by serial in vivo transplantation It was found that the tumours of transfer generations A1 to A5 were characterized by a predominating number of 38 chromosome cells while the succeeding generations A6 to A8 displayed a majority of 37 chromosome cells When tumours from transfer generation A8 were used for continued serial in vivo transplantation and a parallel tissue cultivation procedure different chromosome patterns were obtained

(2) The cultures 8 1 to 8 3 varied widely (from 53 to 73) but no cells were found within the diploid region

(3) The tumours of transfer generations A9 to A11 which corresponded in time to the cultures displayed a majority of cells within the diploid re[illegible]gion and with peaks at 37 A small number of c[illegible] was also found within the 72 to 76 region

(4) When retransplanting cultured cells to [illegible] treated hosts (transfer generation a12) the new o[illegible] grown tumours displayed a modified chromosom[illegible] pattern Thus in comparison with the cultured cel[illegible] split distribution was observed which here indica[illegible] a predominance of 40-chromosome cells in combina[illegible]tion with a wide distribution of cells within primar[illegible] the 53 to 67 region Of special interest is to n[illegible] that while no 40-chromosome cells were found in the analysis of the cultures no less than 51 7 per cent was now recorded In this context it should als[illegible]

Table 4 *(cont.)*

[illegible]	66	67	68/71	72	73	74	75/77
					1	5	1
	3				1		
			1	1		1	1
		1					
				1			
	1	1		2			1

Table 5 *(cont.)*

1	6	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78/99	>100
												1	5	1				
6	15	2	5	1	3							1						
1	1		3	5	4	4												1
				1		2	1	2	3	3	1	3	4	1	1	2	1	1
8	3																	

be mentioned that in this generation no 60-chromosome cells were found in contrast to the cultures which displayed 21 3 per cent

(5) Data obtained from the in vivo transplanted tumours of transfer generation A12 showed a sustained predominance of 37 chromosome cells but also an increased number of 40-chromosome cells Furthermore a few cells were found within the 67 to 77 region

(6) Concerning the succeeding a generations the split chromosome distribution was maintained even if an often marked percentile variation was observed

(7) The A series showed by transfer generation 20 a shift of predominating chromosome number from 38 to 40 and a narrow range of variation It should also be pointed out that sporadic analysis of tumours from later transfer generations (A43–A55) presented a split chromosome distribution but with similarities to the pattern found in the tumours of transfer generations a12 to a25

The results of the in vitro experiment indicate that an artificial environment may drastically affect the chromosome pattern However it seems reasonable to assume that tumour cells were stimulated to grow This opinion is supported not only by the heterogeneous chromosome pattern observed after the cultivation procedure but also by the fact that new tumours of the same histologic type could develop after retransplantation of cultured cells It was also noticeable that these tumours immediately displayed a chromosome pattern somewhat typical of

^{90}Sr induced transplanted tumours i e 40 chromosome cells appearing at different stages of the tumour progression in combination with cells within the triploid region (Part I) This chromosome distribution was also found in the serially in vivo transplanted tumours in transfer generations A43 to A55

SUMMARY

This investigation started from serially in vivo transplanted tumours The chromosome patterns of in vitro cultures and of parallelly and serially transplanted tumours were examined Cultured cells were also used for retransplantation to mice When comparing the chromosome counts of the cultured cells and the in vivo transplanted tumours significant differences were revealed When retransplanting cultured cells it was also noticed that a similar chromosome distribution appeared as previously found in ^{90}Sr induced transplanted series

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AGE AND DOSE RELATED CARCINOGENICITY OF ^{90}Sr

A NILSSON P BIERKE G WALINDER and A BROOMÉ KARLSSON

It seems to be increasingly important to elucidate ow various biologic parameters such as hormonal metabolic imbalances genetic constitution sex d age may influence upon or modify the effect of adiation

As regards age there are quite definite changes in e susceptibility to the induction of various types of mours as a function of the postnatal age at which imals are irradiated (UNSCEAR 1977) It is also own (DECKER et coll 1964 ANDERSON & COMAR 968) that ^{85}Sr is retained maximally when adminis red to dogs of an age of 5 to 6 months i e some hat before puberty whereas when given earlier the tention is reduced largely on account of a high quirement for bone resorption to allow for growth later age a much reduced resorption and accre on was observed Experiments performed by PECKMAN & NORRIS (1964) with mice and rats also early pointed in the same direction Young animals -30 days of age) retained less strontium than oung adults (42–101 days of age) whereas old nimals retained the lowest amount Numerous re orts have stated a relationship between the body urden of ^{90}Sr and frequency of tumour bearing nimals as well as multiplicity of tumours which dicates a strongly age related hazard reaching a aximum just before or during puberty To which xtent this age related susceptibility is also as ociated with a specific sensitivity of the tissues at ifferent ages or only reflects an increased body urden is not well understood Reports by VAN PUTTEN (1969) and FINKEL & MILLER (1964) indi cate that the tissue sensitivity to cancerogenic ef fects of internal emitters under the conditions pre vailing in their experiments does not significantly change with the age of the animal

The main purpose of the present report is to pre sent the mutual connection between injected amount of ^{90}Sr and the age of mice upon factors such as survival frequency of osteosarcomas and lym phoreticular tumours (LR tumours) as well as the incidence of soft tissue tumours

Material and Methods

Three different doses of ^{90}Sr—given intraperito neally as $^{90}Sr(NO_3)_2$—were supplied to 3 series of inbred CBA female mice subdivided into 4 different age groups (Table 1)

The mice were during the whole experimental period kept in the same room and inspected twice daily They were grouped at random and maintained in cages with 10 animals in each A commercial pelleted diet (Standard Feed for Rats and Mice As tra Ewos) and water were supplied ad libitum

Autopsy was carried out when the animals were moribund or in a near moribund state Before autop sy radiography of each mouse was performed in order to detect bone tumours The carcasses were routinely weighed as well as the liver spleen

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Table 1

Experimental design

No of mice	Injection on day	Inj ^{90}Sr/g b w	
		kBq	(µCi)
50	300	29 6	(0 8)
50	150		
50	75		
50	25		
50	300	14 8	(0 4)
50	150		
50	75		
50	25		
50	300	7 4	(0 2)
50	150		
50	75		
51	25		

The asterisks indicate that during the course of the experiment (*) one (*) 2 and ( ) 3 animals were lost

thymus and brachial and axillary lymph nodes These organs all macroscopic hard and soft tissue tumours and radiographically suggested bone tumours were fixed in Stieve s fluid Hard tissues were decalcified in 20 per cent formic acid Ordinary histologic techniques were used and the sections were routinely stained according to the van Gieson method and with haematoxylin eosin

The diagnosis of bone tumours was based upon radiographic post mortem and microscopic findings The number of intraosseous bone tumours recorded is a minimum since only bones with radiologically suggested tumours were examined microscopically All tumours were classified according to the nomenclature preferred by the EULEP (European Late Effects Project Group) Committee on Pathology Standardization

Results

Survival time The age of the animals within the different dose groups had a very small influence on their remaining survival times (Table 2) Thus in the 29 6 kBq group all animals endured approximately 300 days In the 14 8 kBq group the corresponding value was about 400 days except for the mice injected on day 25 which lived significantly shorter than the other age series in the same group ($p<0\ 01$) In the lowest dose group a significant negative correlation ($r=-0\ 997$) was observed between survival times and ages

The bone tumour induction time was roughly the same irrespective of the age at the time of ^{90}Sr administration within both the 29 6 and the 14 8 kBq group In the lowest dose group a probable negative correlation ($r=-0\ 97$ $p<0\ 05$) between induction times and ages was found

Bone tumour incidence The number of tumour bearing mice as well as the total number of tumours are recorded in Table 2 If the osteosarcoma incidence is expressed as the number of tumours per mouse or better the mean tumour induction rate in 50 mice (MTIR Table 2) it is discernible that the age of the animals at the time of ^{90}Sr administration has a great influence on the future appearance of bone tumours The MTIR is defined as the regression coefficient of the tumour frequency in 50 mice as a function of time between T−SD and T+SD where T is the latency time between injection and the clinical appearance of the tumour and SD the standard deviation The MTIR data reveal a highly statistically significant difference of tumours between all age groups irrespective of the dose of ^{90}Sr

Based upon the MTIR data (Table 2) it is evident that the adult 75 day old mice are always more prone to develop tumours Then follow the mice injected on day 25 and on the third and fourth place those given ^{90}Sr on day 150 and 300 respectively It should also be noted that mice given 14 8 kBq/g b w on day 75 belong to the same sensitivity level as mice given 29 6 kBq/g b w on day 300 The same can be observed in mice given 14 8 kBq/g b w on day 150 and those injected with 7 8 kBq/g b w on day 25 and 75 However the mean induction time for the tumours as well as the total number of tumours differ substantially between the different dose groups As could be expected no correlation exists between survival times and total number of tumours even in cases where the MTIR figures are similar

Lymphoreticular tumour incidence The frequency of LR tumours is unrelated to the age of the animals when injected with the nuclide (Table [illegible]) However the two lowest dose levels are much more tumourogenic than the highest one

Other neoplasias Very few soft tissue tumours were observed at the highest dose level irrespective of the age at injection of ^{90}Sr (Table 4) The maximum of soft tissue tumours (70) were found at the [illegible]

Table 2

The incidence of ^{90}Sr induced bone tumours related to age and dose and expressed by the mean tumour induction rate

^{90}Sr/g b w 1 (μCi)	Inject on day	No of mice	Mean survival days±SE	Total No of tumours	No of mice with tumour(s)	Mean induction time (T) days±SE	No of tumours per mouse	MTIR	MTIR level *
6 (0 8)	300	50	272± 9	80	40	293± 5	1 60	0 641±0 032	4
	150	47	312±10	93	42	320± 8	1 98	0 840±0 022	3
	75	48	295± 6	149	47	301± 7	3 10	1 662±0 042	1
	25	50	297± 7	84	40	319± 3	1 68	1 105±0 055	2
8 (0 4)	300	50	413±17	63	35	449±11	1 26	0 332±0 013	6
	150	49	406±18	46	32	490±20	0 94	0 182±0 012	7
	75	49	392±14	113	40	434± 6	2 31	0 624±0 027	4
	25	49	373±24	69	28	340± 7	1 41	0 519±0 014	5
4 (0.2)	300	49	516±21	15	14	572±38	0 31	0 0372±0 0046	9
	150	47	569±27	14	14	667±28	0 30	0 0595±0 0025	8
	75	49	600±23	57	30	683±14	1 16	0 213±0 0059	7
	25	51	625±30	33	24	710±15	0 65	0 133±0 019	7

The mean tumour induction rate (MTIR) is defined as the regression coefficient of the tumour frequency in 50 mice as a function of time between T−SD and T+SD where T is the latency time between injection and the clinical appearance of the tumour and SD its standard deviation

* The level of MTIR given by numbers indicates a highly significant difference ($p<0\ 001$) in MTIR between successive numbers

Table 3

Incidence of lymphoreticular (LR) tumours in relation to age and injected dose of ^{90}Sr

Age group (days)	^{90}Sr dose kBq (μCi)/g b w 29 6 (0 8)		14 8 (0 4)		7 4 (0 2)		Total (per cent)
	No of mice	No of mice with LR tumours	No of mice	No of mice with LR tumours	No of mice	No of mice with LR tumours	
300	50	0	50	7	49	6	13 (8 7)
150	47	2	49	6	48	10	18 (12 5)
75	48	1	49	4	49	9	14 (9 6)
25	50	1	49	8	51	2	11 (7 4)
Total (per cent)	195	4 (2 1)	197	25 (12 7)	197	27 (13 8)	

dose level Of these 41 per cent were confined to the group of animals injected on day 300 If the whole material is taken into consideration the predominance of this group will still be 41 per cent With a few exceptions the main part of the tumours were restricted to the liver uterus ovary lung and mammary gland In the lowest dose group the spectrum of different tumours was more widespread

In the 14 8 kBq group a slight tendency to a higher tumour frequency in the two oldest age groups was found (13 and 9 in the groups injected on day 300 and 150 respectively against 5 and 5 in the two youngest)

The majority of the liver tumours were hepatocellular adenomas (hepatomas) In one case a hepatocellular carcinoma (malignant hepatoma) with metastasis to the lung was found The uterine tumours were either leiomyoma or leiomyosarcoma All ovarian neoplasias were granulosa cell tumours The pulmonary tumours were all classified as al

Table 4

Soft tissue tumours survival time and lifespan in groups of mice injected with different doses of ⁹⁰Sr at 300 150 75 and 25 days of age

Organ with tumour	⁹⁰Sr dose kBq (μCi)/g b w											
	29 6 (0 8)				14 8 (0 4)				7 4 (0 2)			
	Injected on day				Injected on day				Injected on day			
	300	150	75	25	300	150	75	25	300	150	75	25
Liver	–	1	–	–	5*	4	1	1	10	5	3	[illegible]
Uterus	–	1	–	–	2	2*	2	1	5 **	–	[illegible]	1
Ovary granulosa cell tumour	–	–	–	–	3	–	–	–	2	–	1	
Lung alveolar cell carcinoma	–	–	–	–	2	2	–	1	6	2	3	[illegible]
Mammary gland	–	–	–	–	1	1	1	1	–	–	1	
Skin and subcut tissue	1	–	–	–	–	–	–	–	–	4	–	
Harder s gland	–	–	–	–	–	–	–	1	1	–	–	
Adrenal gland Phaeochromocytoma	–	–	–	–	–	–	–	–	[illegible]	–	–	
Spleen haemangiosarcoma	–	–	–	–	–	–	–	–	–	–	2	
Pituitary adenoma ant part	–	–	–	–	–	–	1	–	–	–	–	
Unclassified	–	–	–	–	–	–	–	–	3	–	2	1
Total No of tumours	1	2	0	0	13	9	5	5	29	11	14	16
No of mice	50	47	48	50	50	49	49	49	49	47	49	51
Survival time days M±SE	272±9	312±10	295±6	297±7	413±17	406±18	392±14	323±24	516±21	569±27	600±23	[illegible]
Lifespan days M±SE	572±9	462±10	370±6	322±7	713±17	556±18	467±14	348±24	816±21	719±27	675±23	[illegible]

One malignant hepatoma
Leiomyosarcoma
Four leiomyosarcomas one leiomyoma

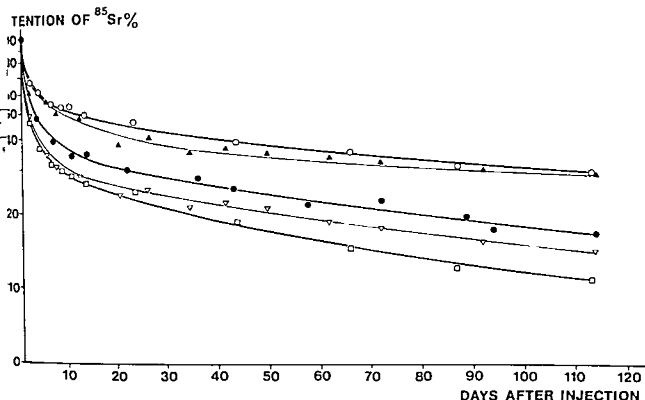
Schwannoma (2) Seb gland adenoma (2)
Adenomas and adenocarcinomas

Table 5

Probable causes of death in groups of mice injected with different doses of ⁹⁰Sr at 300 150 75 and 25 days of age

Inj ⁹⁰Sr/g b w kBq (μCi)	Injected on day	No of mice	Osteo sarcoma	LR tumours	Other tumours	Inanition	Neg autopsy	Other causes
29 6 (0 8)	300	50	34	0	1	10	5	0
	150	47	36	2	1	5	3	0
	75	48	45	1	0	0	2	0
	25	50	33	1	1	14	0	[illegible]
14 8 (0 4)	300	50	25	7	6	6	4	[illegible]
	150	49	26	6	5	4	6	[illegible]
	75	49	37	4	0	1	6	1
	25	49	23	8	4	10	4	0
7 4 (0 2)	300	49	4	6	21	8	9	1
	150	48	8	10	8	12	9	1
	75	49	23	9	8	3	4	[illegible]
	25	51	17	2	9	14	9	0

Emaciation including general hypoplasia of lymphoid and blood forming tissue



tention of ⁸⁵Sr in CBA male mice of different ages (□ 168 days 1?5 days ● 75 days ▲ 50 days ○ 30 days) at the time of exposure in relation to time Each point represents the mean value of 10 animals (After Ronnback)

olar cell carcinomas Those of the mammary ands were either adenomas or adenocarcinomas wo subcutaneous tumours of the chest were hwannomas Both tumours of the adrenal glands ere phaeochromocytomas Six tumours were not ossible to classify on account of autolysis

Causes of death The most probable causes of ath appear in Table 5 In the two highest dose oups the majority of animals irrespective of age at e time of ⁹⁰Sr injection have died from osteosar omas or LR tumours In the lowest dose group an creased number of animals died of other causes ich as soft tissue tumours and inanition particular in the series injected on day 300 In a few cases lso inflammation was found In several animals the ause of death could not be ascertained

Discussion

The MTIR in groups of 50 mice was considered to e more independent of survival times and much ore sensitive and reliable in expressing the osteo arcoma incidence than merely using the number of umour-bearing mice particularly since it is a wellknown fact that the multiplicity of ⁹⁰Sr induced umours is dose related (NILSSON 1970) When comparing the various age series at the three different dose levels the existence of a strong age related sensitivity was evident from the fact that the young adult mice (75 days old) always were more inclined to develop bone tumours than were young mice (25 days old) or mid aged (150 days old) and old mice (300 days old) The reason for this constant finding may above all be associated with the age-determined retention of radiostrontium as has been evidenced by RONNBACK (1979) using ⁸⁵Sr (Figure) and by among others DECKER et coll ANDERSON & COMAR and SPECKMAN & NORRIS

Since no estimations of the radiation dose have been made for the tumour induction in the different age groups it is not possible to express any opinion about variation of the specific sensitivity with age of the bone tissue to the cancerogenic effect of ⁹⁰Sr

The difference of survival time observed between young and young adults on one hand and the old animal group on the other seems largely to be related to the high initial age of the latter when inserted in the experiments In fact the age of these animals (516±300=816 days) is fairly high as compared with the mean life span of female CBA mice (823±22 7 days NILSSON & BROMÉ KARLSSON 1976) The reason for the short survival time of the young mice

in the 14 8 kBq group is difficult to explain but may partly be related to the increased incidence of LR tumours and enhanced rate of inanition including hypoplasia of lymphoid and blood forming tissues partly it may also be associated with the fact that animals which are still growing are more vulnerable to this ^{90}Sr dose (14 8 kBq/g b w) than mice in the other age groups This explanation may have some support by the fact that the induction time for the bone tumours is also significantly reduced among the young animals (t=4 263 $p<0.001$) as compared with the remaining mouse groups in the 14 8 kBq series In the highest dose series this difference will not be recognized because in these series the irradiation dose seems to be equally dangerous regardless of age

No relationship was found between the incidence or LR tumours and age which is in a sharp contrast to the experience when external irradiation is used (KAPLAN & BROWN 1952 JARPLID 1968) The reason for this seems to emphasize previous observations (NILSSON 1971 JARPLID 1974) that ^{90}Sr initiates LR tumours within the bone marrow by a mechanism which differs fundamentally from that in the thymus after fractionated external irradiation

In the present experiments soft tissue tumours were significantly less frequent in the high than in the low level dose series Thus of a total of 105 tumours 67 per cent were found in the low and 30 per cent in the mid level dose Taking into account that the animals in the low level series have been exposed to irradiation for a longer time than in the other series the age seems to be a more decisive factor for tumour development than the dose The mouse groups exposed on day 300 have approximately double the amount of tumours as compared with the groups injected on day 75 and 25 This evaluation also seems to have support from the fact that most tumours occurred in the group with the longest total life span (816 days) i e in the group injected with 7 4 kBq/g b w on day 300 in spite of being exposed to ^{90}Sr significantly shorter (516±21 days) than the group injected on day 25 (625±30 days) at the same dose level

SUMMARY

^{90}Sr was given at three different dose levels (29 6 14 8 and 7 4 kBq/g b w) to groups of miced aged 300 150 75 and 25 days It was found that the incidence of osteosarcomas was highest in the 75-day-group and lowest in the two oldest age groups The frequency of lymphoreticular tumours was inversely dose related (highest incidence in the lower dose series) and not dependent on age at the time of ^{90}Sr injection The frequencies of soft tissue tumours indicate that these tumours are more related to age than to the dose employed

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DOSE DISTRIBUTION AROUND RADIUM ARRAYS USED IN THE TREATMENT OF UTERINE CARCINOMA

P D SHARMA and J C F MACDONALD

The determination of absorbed dose in gynecologic applications of brachytherapy sources has always been more difficult and less accurate than in any other branch of radiation therapy This difficulty is inherent in a technique which involves using linear sources at short distances where the inverse square law gives rise to large dose gradients Despite the advent of the electronic computer and the consequent improvement in the accuracy of calculated dose distributions still many uncertainties are involved not the least the positions and shapes of relevant organs

HEYMAN (HEYMAN et coll 1941) introduced the so-called packing technique in which the uterus is packed to capacity with a number of spacing filters enclosing radium tubes When the technique is properly executed the uterine wall is fixed relative to the radium so that when the exact location of the tubes is determined it is possible to deduce an isodose distribution applicable to the size and shape of the uterus in question Stereoscopic films are helpful in the localization of the tubes but they are only the first link in a long chain of tedious procedures that must be performed before an accurate dose distribution can be produced

BENNER (HEYMAN et coll 1941 HEYMAN & BENNER 1946) considered that the resultant dose distribution should not be critically dependent upon the positions of the radium sources and for this reason he placed little emphasis on exact localization procedures On this assumption he produced a set of tables linking the number of sources to an optimum treatment time which was based on an estimation of resultant dose rates at 1 5 cm from the uterine wall From a modern dosimetric viewpoint this approach is unsatisfactory since the method used for treatment time calculation involved no correction for self absorption among the tubes themselves nor any allowance for the shape of the array In addition the type of filter originally used by HEYMAN bears little resemblance to that commonly in use today A comparison of two Heyman applicator filters with a modern Campbell type stainless steel filter is made in Fig 1 It is seen that these are considerably different in physical dimension and so one would expect the result in dosimetry to be considerably different

In his measurements BENNER used ionization chambers to determine the dose at short distances from the array Because of the large size of these detectors the spatial resolution was poor All measurements were made on the surface of a simulated uterus in air and doses were only recorded at a fixed distance from the radium which was assumed to correspond to the outside wall of the uterus

In the present work the following problems were analysed (1) what the uterus receives during a typical insertion of present-day applicators and (2) how valid the tables originally deduced by BENNER are when applied to existing techniques

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Table 1

Comparison of experimental and theoretic values for a single 10 mg radium tube filtered through 1 mm of platinum Source diameter 0 29 mm active length 1 25 cm

Experimental points as defined by YOUNG & BATHO X	Y	Calculated values from YOUNG & BATHO (mGy/h)	Experiment (mGy/h)
0	1	649	629
0	2	176	169
0	3	79	73
0	4	44	47
0	5	28	32
0	6	19	21

X is the displacement of the perpendicular from the center of the active length of the source
Y is the perpendicular distance of the point from the axis of the source

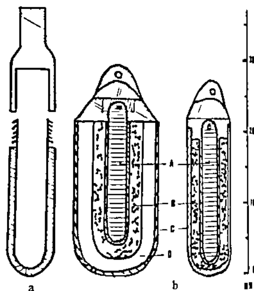


Fig 1 Comparison of a) Campbell type applicator and b) Heyman irradiators A Radium tube B Inner wall of lead C External wall of stainless steel D Intermediate space filled with air

In examining these problems measurements were made using teflon coated 1 mm × 6 mm lithium fluoride thermoluminescent microrod dosimeters these were employed because of their small size and good spatial resolution The measured response of these detectors is a function of their own structure and of the readout device It is therefore necessary to perform an absolute calibration at the time of each measurement to eliminate any short term variation in the sensitivity of the system

Calibration

Thermoluminescent LiF microrods manufactured by the Harshaw Chemical Company were used for the dosimetric determinations

The energy dependence of these dosimeters in the photon energy range up to 1 25 MeV is shown in Fig 2 It appears that between E = 137 keV (3 mm of Cu HVT) and 1 25 MeV (15 mm of Cu HVT) the maximum variation in response is 5 per cent At lower energies the energy dependence is significant with an enhanced response of up to 40 per cent relative to the response at 1 25 MeV

The overall reproducibility of measurement using these detectors was found to be adequate for the present purposes Sets of 20 measurements at each of a number of dose levels produced a standard deviation of 5 per cent In the first 48 hours after irradiation a drop of about 10 per cent in response occurred after which the dosimeter showed no further significant fading with time Accordingly the microrods were calibrated by irradiation to a known dose of ^{60}Co radiation and the practice of reading the dosimeters two days after irradiation was instituted The justification for using these dosimeters is that in sharp dose gradients the positional accuracy is a limiting factor For example at 2 0 cm from radium tubes the dose rate varies by about 10 per cent per mm An estimate of the reproducibility of this system is ± 1 0 mm so that an overall accuracy of ± 15 per cent is all that can be expected

The experimental method was first checked by evaluating the dose distribution in water from a single 10 mg radium tube A polystyrene plate immersed in a water phantom held the radium source

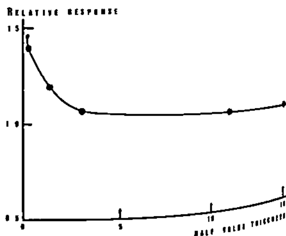


Fig 2 Energy dependence of LiF microrods

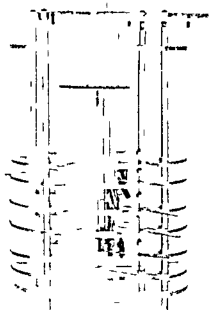


Fig 3 Source and detector jig



Fig 4 a



Fig 4 b

Fig 4 a) A p film of the actual packing b) Control film of the source support

centered in a polar array of lithium fluoride microrods The measured dose rates at various distances were compared with the values calculated from BATHO s tables (BATHO & YOUNG 1964 YOUNG & BATHO 1964) The results are compared in Table 1 It is seen that at distances greater than 4 cm the measured values are high by an amount greater than the experimental uncertainty The contribution of low energy scattered radiation at these distances together with the increased sensitivity of the dosimeter for low energies could give rise to this increased response Lithium fluoride microrods were thus found suitable for measurements under water

Apparatus

A 45 cm × 45 cm × 40 cm deep tank made of clear plastic and filled with water was used for the measurements The source and detector supports (Fig 3) were made of water equivalent plastic and were rigidly fixed in relation to each other so that any arrangement could be accurately reproduced The detector support consisted of six plastic rings mounted on four uprights fitted into a shallow recess in the bottom of the tank These uprights were graduated so that the vertical position of the rings could be recorded and reproduced Each ring carried six polystyrene strips spaced around it at intervals of 60 degrees The lithium fluoride rods were fitted into slots in the strips Each strip could move in the slot and was graduated so that its radial position could be reproduced

In order to hold the radium sources in position a support (Fig 3) fitting tightly into the top of the detector jig was constructed The support was made up of two spaced plastic plates into which a matrix of holes was drilled Polystyrene rods bent to any desired shape by heat treatment were then pushed through the appropriate holes and clamped in position Thin walled polystyrene holders were attached to the ends of these rods In this way it was possible

to reproduce virtually any clinical radium distribu tion the presence of the polystyrene rods support ing the lower sources would occasionally interfere with the exact orientation of the upper sources

Experimental procedures

A p and lateral films of eight typical Heyman treatments form the basis of the reconstructed radium arrays The polystyrene support rods were adjusted to produce as nearly as possible a model of the actual packing arrangement The accuracy of this procedure was controlled by exposing films of the source support with the dummy sources in place (Fig 4)

Microrod dosimeters were distributed on the de tector strips the strips were mounted on the rings and then pushed into their final positions Campbell applicators (a modern version of the Heyman filters) each containing a 10 mg radium tube were then placed in the holders in the source support which was then inserted in the detector array Thereafter this entire arrangement was immersed in the water tank and left for an irradiation time of 20 to 24 hours the time depending upon the number of sources used During this period films of the arrangement were exposed in two directions at right angles the geometry being chosen to make the magnification correspond to that of the original films of the patient With the radium removed the dosimeters were read together with the calibration detectors using a Tele dyne TLD 7300 reader From a graph of dose rate against position along each strip the dose rate dis tribution at the level of each ring was determined From the distributions in the six horizontal planes dose rate distributions in the other two mutually perpendicular planes could be produced by interpo lation By using the known magnification factors it was possible to transfer the known dose rate con tours directly onto the original films

The computation of the dose distributions was performed using an Artronix PC 12 computer and employing as input the coordinates of the ends of the applicators as determined from measurements on pairs of orthogonal films This straight forward cal culation was complicated by problems of cross fil tration frequently the dose at a point from a particu lar source would be reduced because of absorption by one or more other source capsules In addition the extra filtration provided by the spacing filters had to be taken into account in the calculations The

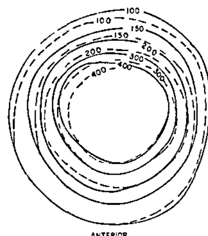


Fig 5 Dose distribution (in mGy/h) for the 5 tube array L measurement ( ) Computer calculation (—)

contribution of these factors was measured exper mentally using thermoluminescent dosimeters F two Heyman encapsulated radium tubes the cros filtration effect was determined to be 3 per cent a 2 5 cm distance and the effective absorption fro the filters was 4 per cent In order not to compli a the computer calculations a constant absorpt factor of 8 per cent for each filter was employed

The results of the computer calculations and the experimental measurements for a particular ar ray in a specific plane appear in Fig 5 In this case it may be concluded that the agreement is within th region of the combined error of the two methods total of 8 arrays ranging in activity from 5 to 16 1 mg radium tubes were analysed In each case comparison of the results of dose measuremen with those of computer calculations were compar with values derived from BENNER s tables Th comparison at a distance of 15 mm from the extern surface of the radium a distance which is consid

Table 2

Experimental measurements of Heyman arrays

No	Number of Ra tubes	Measurements at 1 5 cm (mGy/h)	Calculated from BENNER s tables (mGy/h)	Comput… cal… (mG…
1	5	394±60	458	431
2	5	576±80	458	463
3	6	459±70	500	441
4	6	356±50	500	361
5	8	468±70	565	484
6	8	493±70	565	[illegible]
7	12	696±100	700	[illegible]
8	16	880±170	804	[illegible]

red to be equal to the average thickness of the terine wall is shown in Table 2

Accuracy In the radiation measurements with a producibility of source and detector of approxi mately ± 1 0 mm an overall dose determination accuracy of ± 15 per cent is expected

In the computer calculations the accuracy of the computer dose rate depends upon the accuracy of determination of the end points of the sources from measurements on the films This again is estimated to be ± 10 per cent Other sources of uncertainties ly in the assumption of the effects of cross filtration and in the variation in the true activity content of the tubes The overall effect of these variables leads to an expected accuracy of ± 15 per cent

Conclusions

The HEYMAN packing method from its inception has been recognized as a system that is not amena ble to accurate dosimetry In his pioneer work BENNER recognized this and on the basis of ex periments that would be considered crude in today s standards recommended a method to form a routine basis for dose evaluation

Computers at present available make possible the determination of dose at any point in the vicinity of a radioactive array the principal problem is in many cases the specification of significant points at which the dose should be determined In the packing technique BENNER s points of interest 15 mm beyond the outer most radium may still be consid ered to be a reasonable region of interest

In the present work measurements have been made to determine the dose distribution around eight typical packing method arrays of commercially available Heyman applicators A comparison of the results of these measurements with the dose rates derived from BENNER s tables shows satisfactory agreement even when applicators are employed that are considerably different from those used by HEYMAN

Further it has been established that standard dosimetric computational techniques produce results which are in agreement (within acceptable levels) with those of the results of measurement and with the values of BENNER

This work supports BENNER s approach to the Heyman technique in that a reasonably uniform dose distribution can be achieved from a haphazard array of tubes and that this dose distribution is insensitive to small changes in techniques

SUMMARY

Measurements have been made to determine the dose distribution around typical arrays of commercially avail able Campbell type Heyman applicators using ther moluminescent dosimeters These measurements verify the tables produced by BENNER linking the number of sources used in the so called packing technique for the treatment of uterine carcinoma to an optimum treatment time

ACKNOWLEDGEMENT

This work was supported by a research grant from The Ontario Cancer Treatment and Research Foundation Pro ject Numbers 282 and 297 which is greatly acknowledged

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ROM THE LABORATORY OF EXPERIMENTAL BIOLOGY DEPARTMENT OF ANATOMY THE DEPARTMENT F RADIATION THERAPY UNIVERSITY OF GOTHENBURG S-40033 GOTHENBURG AND THE INSTITUTE FOR APPLIED BIOTECHNOLOGY S-43122 MOLNDAL SWEDEN

IRRADIATION INJURY OF BONE TISSUE

A vital microscopic method

T ALBREKTSSON M JACOBSSON and I TURESSON

Numerous reports (for review see NATHANSSON 977) deal with the effect of irradiation on bone ssue but many central questions remain unsolved s for instance whether bone necrosis after irradia ion is preceded by a vascular injury Histology and ucroangiography after irradiation are indirect nethods and provide no repeated evaluation of the vents occurring in the injured bone Only a direct nethod also enabling a repeated observation of he same tissue compartment at different stages after defined irradiation dosage would allow more lefinite conclusions concerning the effect of irradia ion The titanium bone chamber is an attempt to vercome drawbacks connected with the indirect nethods Using the bone chamber vital microscopy of bone tissue and vessels becomes possible both efore and at repeated intervals after irradiation Direct comparison can thus be performed and the levelopment of the tissue injury can be recorded in itu Previous vital microscopic methods applied to he examination of soft tissues such as the rabbit ar chamber (SANDISON 1924) or the mouse skin hamber (ALGIRE 1943) have also been used on rafted bone tissue (SANDISON 1928 KIRBY SMITH 1933 CLARK & CLARK 1942 KIEHN et coll 1952 EZRA COHN et coll 1969 SUDMANN 1975) These methods although allowing a repeated observation of bone tissue changes in the living animal are limited to heterotopical bone grafts No evaluation of the behaviour of bone tissue in situ is possible However this can be performed using the titanium bone chamber now described

Material and Methods

Bone chamber and recording procedures The titanium bone chamber (Fig 1 a) consists of a hollow screw into which one short and one long glass rod are glued leaving a 100 micron wide space in be tween If the chamber is inserted into a long bone of a rabbit using a gentle surgical technique bone and vascular tissue will grow into this space during a healing period of 4 to 6 weeks With the animal in light anaesthesia it is then possible to observe the ingrown tissue in the vital microscope The bone and vascular structure are recorded on film and slides

Vital microscope A modified Leitz intravital microscope was used for all in vivo recordings As a rule magnifications between 20× and 100× were tried A Vinten scientific 16 mm film camera and a Leica camera were connected to the vital micro-scope Kodachrome 25 cine film and Ektachrome 200 film were used

Animals and anaesthesia Preliminary experi ments were performed on 5 healthy adult rabbits (Belgian hare) 10 to 16 months of age weighing 4 5 to 6 0 kg During surgery irradiation and vital

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microscopic sessions the animals were under general anaesthesia maintained by intramuscular injections of Hypnorm (Mekos Helsingborg Sweden) at a dose of 0 7 ml/kg body weight The Hypnorm drug is a combination of Fentanyl and Fluanison

Irradiation procedure The rabbits were irradiated at a temperature of 22 C During the irradiation the bone of the animal was placed upon a 10 cm thick polystyrene phantom A ^{60}Co unit (Siemens Gamma tron G3) was chosen to minimize the difference in the absorbed dose in soft tissue and bone and in the disturbances in the radiation field due to the titanium chamber Source skin distance was 60 cm and the field size was 5 cm × 5 cm The dose rate was 1 20 Gy/min A 5 mm bolus of silicone rubber material was applied to ensure full build up as the top of the bone chamber was only 2 mm below the skin surface (Fig 2) Each rabbit was given 15 0 Gy (the absorbed dose in water at 5 mm corresponding to the position of the chamber) The appearance of the bone was recorded during the healing period following surgery and 2 weeks later The irradiation was then performed (Fig 1b) Further recording was performed each fourteenth day until the animal was killed

Histologic technique After killing of the animal fixation of the bone in formaldehyde and decalcification in formic acid the tissue in the chamber space was removed embedded in paraffin cut transversally and stained with HTX eosin

Results and Discussion

The woven bone before irradiation (Fig 3 a) became subsequently lamellated (Fig 3b) This process involves reorganization of the collagen fibres and the osteocytes of the bone (ALBREKTSSON 1980) In principle woven bone maturation does not seem to be influenced by the irradiation at least not during the first weeks At about 4 weeks after the irradiation (Fig 3 c) the mature bone tissue was slightly remodelled as demonstrated by the changed appearance of the islands of connective tissue proper in the bone This bone remodelling occurred at a similar rate and to the same extent as in normal bone in a series of 70 non irradiated rabbits (ALBREKTSSON 1979) At 6 weeks after injury (Fig 3 d) the vascularity changed and a number of wide venular vessels appeared in the bone tissue Bone

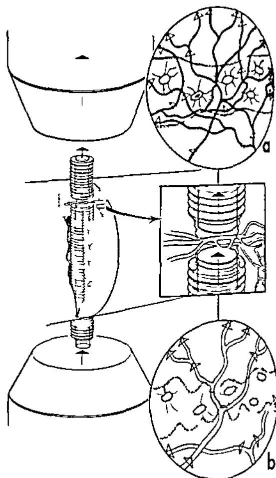


Fig 1 a) Tissue grown into the chamber space (enlarged ... tion) before irradiation The rounded drawing is a sche... representation of the chamber microscopic image ... osteocyte is thought to represent a nutritive bone unit b) Re... intravital recording at 2 weeks after irradiation

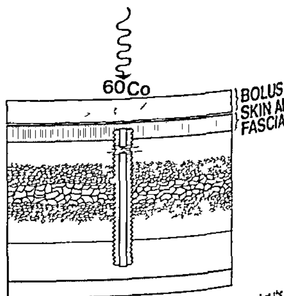
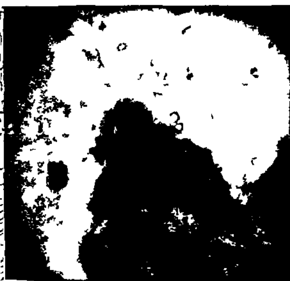
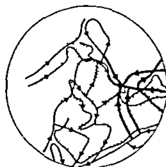


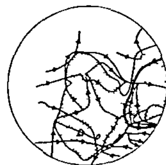
Fig 2 A 5 mm bolus of silicone rubber was applied at the ... diation



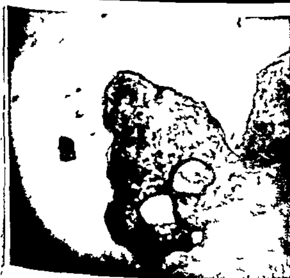


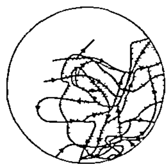
a) Conditions before irradiation Osteogenesis is indicated by woven bone in some places





b) 2 weeks after irradiation The former woven bone in the left part of the bone tongue is now mature as indicated by a clear outer demarcation line and normal osteocytes





c) 4 weeks after irradiation all bone in the chamber is considered mature Up till now the remodelling of the bone is quite normal

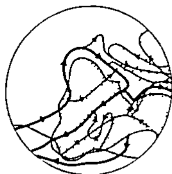
Fig. 3 In the explanatory sketch close dots represent mature bone and spaced dots woven or immature bone Arrows on vessels indicate direction of blood flow 15 Gy were given at irradiation





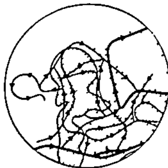
d) 6 week wide venule immature bone appears again and [illegible] active bone resorption starts





e) 8 weeks after irradiation bone resorption is widespread and immature bone is further developing





f) 10 weeks after irradiation most of the original bone is [illegible] The chamber image is instead dominated by immature bone [illegible] with no outer demarcation or a clear representation of [illegible] lacunae

Fig 3 (For legend see preceding page)

sorption began At 8 weeks (Fig 3 e) resorption f the original bone was more apparent and at 10 eeks most of the original bone was resorbed The ndings of wide venulae appearing before beginning f bone resorption should not be considered as im ying that the injury to the vessels occurred more pidly than that to the bone It has previously been oserved (ALBREKTSSON 1980) that bone resorption ormally is associated with wide vessels and slow lood flow This means that the wider venulae obrved might just as well reflect a vascular response abnormal bone as a primary vascular injury due irradiation Simultaneously with the onset of bone sorption the development of new bone tissue was bvious (Fig 3d right part of the observation eld) This new bone tissue rapidly spread over the hamber field during the following weeks (Fig e-f) The vital microscopic view of this bone sembled that of woven bone which however ormally would become lamellated during a time pse of 6 weeks This new bone still lacked definite uter demarcation and clearly established osteocyte cunae at 10 weeks

The present experiments have shown that the tanium chamber is a well functioning method for bserving and recording bone injury following irra iation The vital microscopic approach allows a emonstration of the different stages in the development of an irradiation injury The method has been pplied in further experiments on the effects of fferent types of irradiation injury to bone tissue

SUMMARY

A vital microscopic method using the titanium chamber r observation of bone irradiation injury in situ is scribed The subsequent development of bone tissue resorption and replacement with a pathologic immature bone could be observed and recorded

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ACTA RADIOLOGICA

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ONCOLOGY

1980

THECA-CELL TUMORS

Clinical features and prognosis

ELISABET BJÖRKHOLM and CLAES SILFVERSWARD

Theca cell tumors thecomas were first described LÖFFLER & PRIESEL (1932) The crude annual idence among Swedish women in the period 1958 1972 was 0 74 per 100000 (BJÖRKHOLM & FVERSWARD 1980) Thecomas belong to the anulosa stromal cell tumor group which consists neoplasms containing granulosa cells theca cells d stromal cells resembling fibroblasts singly or in nous combinations Pure theca cell tumors are nost always benign (SCULLY 1970 NORRIS & IORLTON 1974) whereas granulosa cell or mixed anulosa theca cell tumors may be malignant IORRIS & SCULLY 1958) Thus it is important to ntify patients with pure thecomas who should be ated with surgery alone not including them in the anulosa cell tumor group in which in certain cases adiation may be added Women with thecomas nd granulosa-cell tumors) often have endometrial rcinoma (INGRAM & NOVAK 1951 MANSELL & ERTIG 1955) Sixty two patients with thecoma ne been treated at Radiumhemmet one fifth of em having concomitant endometrial carcinoma he clinical features and the treatment of these omen are now presented The survival has been etermined by comparison with age and geographi ally matched control women

Material and Methods

During the period 1923 to 1972 313 women with ncroscopically confirmed granulosa stromal cell imors were treated at this oncologic department Material from the ovarian tumor (the original slides or new slides prepared from the original paraffin blocks and stained with haematoxylin-eosin) was still available for 278 cases A re evaluation was per formed the clinical data being unknown to the pathologist (C S)

The present series ultimately consisted of 62 cases considered to be pure thecomas Endometrial biop sies dating from time of diagnosis of the ovarian tumor were available for 52 per cent of these cases The diagnosis thecoma was consistent with the ini tial diagnosis in 39 cases 3 cases originally dia gnosed as thecoma were now being considered as granulosa cell tumors as they contained epithelial strands of the granulosa cell type and 17 cases pre viously diagnosed as granulosa cell tumors were now considered to be thecomas Six fibromas were at review included in the thecoma group because of typical theca cell proliferation some of these also with signs of estrogenic function (the patients having glandular cystic hyperplasia of the endometrium) Clinical data were collected from the hospital re cords The system of civil registration in Sweden made it possible to obtain age and geographically matched control women limited to the patients re siding outside Stockholm City Forty control women were assigned to 65 per cent (20) of the women with theca cell tumors living outside Stockholm City at

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the time of diagnosis The patient and her 2 controls were of the same age when they entered the series at the time of diagnosis of the patient s ovarian tumor Information on civil status and childbirth for both groups was obtained A follow up was performed in 1977 and 1978 A final date for collecting the series was set to 25 November 1977 Causes of death were based on information from death certificates Survival over time was described with life tables and differences in survival were tested using the log rank test (PETO et coll 1977)

Results

The mean age at diagnosis was 59 5 years range 19 to 81 years (Fig 1) and 84 per cent of the women had passed the menopause A history of abdominal pain of varying severity was given by one third of the patients Abnormal uterine bleeding was reported by 6 of 10 pre menopausal women and one patient 23 years of age had experienced secondary amenorrhoea during one year before diagnosis Among post menopausal women 60 per cent had reported uterine bleeding All patients belonged to clinical stage I (FIGO) 3 per cent had bilateral tumors and 5 per cent had ascites exceeding 0 5 liter The mean tumor size was 7 cm range <1 to 20 cm Thirteen endometrial carcinomas 21 per cent of the total series were confirmed at review 8 were highly differentiated adenomatous carcinoma 4 were differentiated adenomatous carcinoma with partly solid areas and 1 was a predominantly solid carcinoma (FIGO) The mean age at diagnosis of the women with a concomitant endometrial carcinoma was 69 8 years range 47 to 71 years One case of atypical hyperplasia was diagnosed Glandular cystic hyperplasia was found in 56 per cent of the reviewed endometrial biopsies The hormonal changes of the endometrium were impossible to observe in one third of the cases as all endometrial tissue was malignantly transformed The contralateral ovary was available in 12 cases one third having ovarian stromal hyperplasia All patients were operated upon (Table) Additional radiation therapy was given to 60 per cent of the patients of these 16 per cent had a concomitant endometrial carcinoma At the follow up the mean observation time was 15 5 years range 1 to 34 years Thirty two women had died and no patient was lost to the follow up No patient with thecoma died from the ovarian neoplasm Malignant disease caused death in 7 women

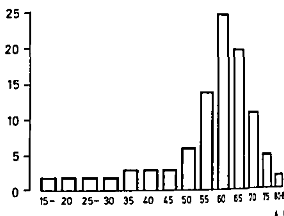


Fig 1 Distribution of 67 patients with thecoma by age (years)

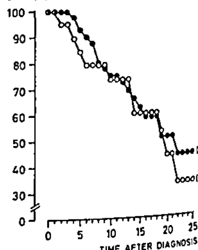


Fig 2 Crude cumulative survival (per cent) at time after diagnosis (years) The numbers of patients (O) and controls (●) at entry were 20 and 40 respectively still living and under observation after 25 years were 1 and 3

3 endometrial carcinomas one mammary carcinoma and 3 intestinal tumors

The crude cumulative percentage of survival (counting deaths from all causes) for the 70 patients with thecoma and their 40 controls is given in Fig 2 half of the patients had received radiation therapy of the 20 patients had a concomitant endometrial carcinoma No statistically significant difference in survival was found between the patient and the control group The mortality from cerebrovascular and cardiovascular diseases dominated and was equal for both groups Endometrial carcinoma caused death in 2 women of the patient group whereas a control woman died of that cause Of married women in the patient group 71 per cent had given birth

Table

Sixty-two patients who at review were classified as thecoma by original diagnoses and mode of treatment

Mode of treatment	Original diagnosis			Total No of cases
	Thecoma	Granulosa cell tumor	Fibroma	
Unilat oophorectomy	4	4	0	8
Unilat oophorectomy and radiation therapy	2	1	0	3
Bilat oophorectomy	5	1	3	9
Bilat oophorectomy and radiation therapy	10	6	3	19
Hysterectomy and bilat oophorectomy	5	2	0	7
Hysterectomy bilat oophorectomy and radiation therapy	3	0	0	3
Treatment for concomitant endometrial carcinoma Hysterectomy and bilat oophorectomy with or without radiation therapy	10	3	0	13
Total	39	17	6	6?

at least one child at time of diagnosis at that time corresponding figure for the control group was per cent

Discussion

A thecoma is generally easy to identify at micro ...opy It is composed of pale oval or spindle ...aped cells arranged in irregular interlacing bun ...s traversed by bands of fibrous tissue with var ...ng degrees of hyalinization Fat droplets are dem ...strable by special stains in and between the ...lls It may sometimes be difficult to distinguish ...comas from sarcomatoid granulosa cell tumors ...anulosa cell areas not clearly identifiable on ...ematoxylin-eosin stained slides may be present in ...me thecomas (WAXMAN et coll 1979) Silver re...ulum stains have been used to try to identify ...anulosa and theca cell components The theca ...lls are individually surrounded by this reticulum ...hereas granulosa cells are not at all enclosed or as ...oups of cells (TRAUT et coll 1939) The reticulum ...ained slides are sometimes confusing and difficult ... evaluate (KNIGHT 1948) nor do they always re... this network (BIGGART & MACAFEE 1955)

No serious differential diagnostic difficulties were ...countered when reclassifying this series In one or ...o cases a silver reticulum stain might have added ... the diagnostic accuracy but as only the original ...des were accessible and no paraffin blocks a...er reticulum stain was impossible to perform in ...ose cases

It is impossible to draw a sharp demarcation line between fibromas and thecomas (MORRIS & SCULLY) They may indeed be variants of a single neoplasm with a common origin from the ovarian stroma (AMIN et coll 1971) The thecoma is typically estrogenic the fibroma is non functioning In this series fibromas were defined as small tumors with markedly few cells of the spindle cell type and with an abundance of collagen Fat is usually not demonstrable in fibromas

The clinical features of the patients with thecoma in the present series are similar to those previously reported (BANNER & DOCKERTY 1945 STERNBERG & GASKILL 1950) A high proportion of endometrial carcinomas was found in patients with this tumor Feminizing ovarian tumors (granulosa and theca cell tumors) are often complicated by endometrial carcinoma However only 0 4 per cent of the patients with endometrial carcinoma treated at Radiumhemmet between 1923 and 1972 had a concomitant feminizing tumor 0 2 per cent were of the thecoma type (BJORKHOLM unpublished data) Almost all thecomas are benign but a few malignant cases have been reported A review of the literature has recently been published by WAXMAN et coll who proposed that the name malignant thecoma should not be used Instead they suggested the name stromal sarcoma for those very rare malignant stromal ovarian neoplasms that are composed of primitive mesenchymal cells fibroblasts and theca cells

No woman in the present series died from the

thecoma No difference in survival was found be tween the 20 patients and their 40 controls although more than one third of these patients had a con comitant endometrial carcinoma which in the majority of the cases was highly differentiated Cerebrovascular and cardiovascular disease was the major cause of death in both patients and controls

WYNDER et coll (1969) did not find any real dif ferences between patients with ovarian malignancy and controls as regards age at first pregnancy and delivery last pregnancy and delivery and total number of pregnancies The number of parous mar ried women was almost the same for the present patients and the controls

It is noteworthy that half of the patients without endometrial carcinoma received complementary radiation therapy due no doubt to a previous un certainty as to whether these neoplasms were of a malignant nature or not To day unilateral oophorectomy in pre menopausal women with a stage Ia (FIGO) disease would be suggested The possibility of a concomitant endometrial carcinoma has to be ruled out especially for post menopausal women No complementary irradiation should be given because of the ovarian tumor

SUMMARY

During the period 1923 to 1972 62 women with theca cell tumors were treated at Radiumhemmet The mean age at diagnosis was 59 5 years Concomitant endometrial car cinoma was found in 13 patients At 1978 after a mean observation time of 15 5 years 30 patients were alive No patient died from thecoma Malignant disease caused death in 7 women No difference in survival was found between 20 patients and 40 controls matched by age and place of residence

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.ENSITIVITY OF CELLS IN EXPONENTIAL AND STATIONARY GROWTH 'HASE TO COMBINED TREATMENT WITH RADIATION AND QUINACRINE

J MIDANDER and B LITTBRAND

Quinacrine has been shown to inhibit repair of liation induced DNA single strand breaks in bac ia and in mammalian cell lines (FUKS & SMITH 71 MODIG et coll 1974 VOICULETZ et coll 1974) the case of bacteria the inhibition of repair could related to a decreased recovery of the cells from blethal radiation injury but in the case of a mam llian cell line data from survival curves did not licate such a correlation (VOICULETZ et coll) In ler to elucidate further the question of a correla n between DNA injury and radiation survival af Quinacrine treatment in addition to investigat the radiation survival of the cells also their overy during a split dose interval and the survi l in a particular low dose range was examined A 'ect quantitative determination of the recovery 'ead of estimating it indirectly by extrapolation is supposed to give valuable information of the chanism by which Quinacrine acts as a radiation nsi izing agent Cells both in exponential and tionary growth phase were used in order to obtain ell material with a varying potential for recovery AHN 1968)

Materials and Methods

A substrain of the Chinese hamster cell line (V79) as used propagated under standard tissue culture nditions The nutrient medium consisted of agle s medium in Earle s saline supplemented with 10 per cent fetal calf serum and antibiotics For each experiment monodispersed cells derived from 1 day old cultures (cells growing in the exponential phase) or 7 day old cultures (cells growing in the stationary phase) were explanted in known numbers in Anumbra Petri dishes (Ø 5 cm) and incubated in a CO incubator for 3 hours Quinacrine dihydro chloride (from Sigma Chemical Co) dissolved in the culture medium was used In the irradiation experiments treatment with Quinacrine was made as a rule at a concentration of 10 μg/ml for 30 min before and during irradiation After irradiation the substance was removed by washing the cells twice with trypsin buffer and reincubating them in fresh medium In split dose experiments Quinacrine treat ment was given on both radiation exposures

The cells were irradiated at room temperature attached to the Petri dishes and with medium re moved to such an extent that only a total of one ml was left The dishes with cover removed were placed in a plastic radiation chamber flushed with pure oxygen supplemented with CO_2 to maintain pH 7 2 After irradiation the dishes were refilled with fresh medium and incubated for 8 days with one medium change on the second day The clones which developed were fixed and stained in situ thereafter the mean number of clones was estab lished by counting three replicate dishes The frac

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tion of cells which survived irradiation was expressed as the percentage of the non irradiated controls in the same experiment The plating efficiency was defined as the percentage of non irradiated cells which grow into macroscopic colonies in 8 days

Radiation was generated with a Siemens Stabilipan Roentgen Unit at 220 kV and 15 mA The half value layer was 0 3 mm Cu and the dose rate was 3 8 Gy/min at the bottom of the culture dishes at an FD of 40 cm The dose delivered was measured by a Philips integrating dosemeter

Results

Toxicity tests In preliminary experiments the cellular toxicity of Quinacrine was analysed with clonogenic survival as end point One hundred cells from exponentially growing cultures were plated in a series of Petri dishes After incubation for 3 hours one ml of Quinacrine in different concentrations and dissolved in culture medium was added to the dishes The cells were incubated with the substance for periods varying between 10 and 120 min then the cells were washed twice with trypsin buffer and reincubated for 8 days Fig 1 indicates that at a concentration of 5 μg/ml Quinacrine had only a slight toxicity up to 60 min at 10 μg/ml the toxicity was slight up to 30 min while at higher concentrations (20 and 40 μg/ml) severe toxicity was apparent already after 20 min

Radiation survival curves The effect of Quinacrine (10 μg/ml) on the survival of cells in stationary growth phase was analysed in 8 replicate experiments each paired with Quinacrine untreated controls Survival determinations were made with 3 different radiation doses which gave surviving fractions below 35 per cent in order to avoid the shoulder region Quinacrine treatment changed slightly the D_0 but no change occurred in the extrapolation number (n) of the survival curves (Fig 2) In 6 similar replicate experiments with cells in the exponential growth phase and irradiated with 4 different doses a slight change in the D_0 was noted but a considerable decrease of n (Fig 3)

The data presented in Figs 2 and 3 suggest that Quinacrine in the concentration used inhibits the recovery from sublethal radiation injury in cells in their exponential growth phase Cells in their stationary growth phase known to have a reduced capaci-

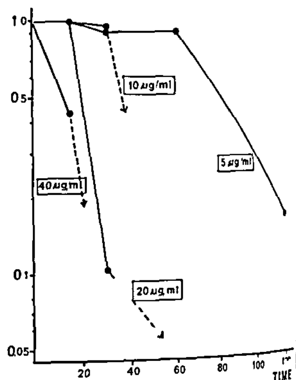


Fig 1 Survival of Chinese hamster cells after exposure to Quinacrine in different concentrations for varying times (min) Means are illustrated from triplicate determinations

ty to recover (HAHN 1968) are not detectably inhibited by Quinacrine

Low dose experiments The conclusion drawn was confirmed in 2 types of complementary experiments In one of them cells were irradiated with relatively low doses to obtain survival in the shoulder region of the survival curves In 9 paired experiments performed with cells in the exponential growth phase and in 7 paired experiments with cells in the stationary phase the survival of Quinacrine treated and Quinacrine untreated cells was determined and the survival ratio calculated The survival ratio for the cells in the exponential phase increased from a value close to unity up to a value near 2 with doses from 0 65 to 2 60 Gy (Fig 4) For cells in the stationary phase only a slight increase was noted from unity to about 1 2

Split dose experiments In another series of replicate experiments the split dose ratio of the Quinacrine treated and Quinacrine untreated exponential or stationary phase cells was calculated The cells were irradiated either with a single dose of 7 80 Gy or with 3 90 Gy on 2 occasions separated by interval of 18 hours Previously this time was

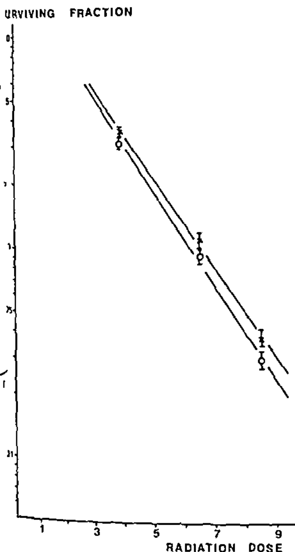


Fig ? Survival of cells irradiated (Gy) in the stationary growth phase after treatment with 10 µg/ml Quinacrine Means ± SE are illustrated from 8 replicate Quinacrine treated experiments (O) and with Quinacrine untreated controls (X) Regression lines were calculated by least square analysis of the pooled data D was 1 94 and 2 05 Gy and n was 2 47 and 2 47 for the Quinacrine treated and Quinacrine untreated cells respectively

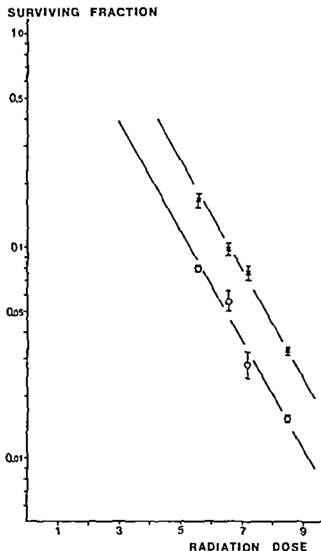


Fig 3 Same as Fig ? but the cells were irradiated in the exponential growth phase D_0 was 1 69 and 1 79 Gy and n was 2 3 and 3 8 for the Quinacrine treated (O) and Quinacrine untreated (X) cells respectively

ound to be less than the time necessary to complete cell division under the conditions used (LITTBRAND 1970) Only a slight decrease in the y of cells in the stationary phase occurred during this time interval (Fig 5) In contrast Quinacrine inhibited considerably the recovery in the exponential phase cells

Discussion

The 3 types of irradiation experiments are consistent in indicating that recovery of the cells from sublethal radiation injury is inhibited by Quinacrine when the cells were treated in their exponential growth phase exhibiting a large recovery potential When the cells had been treated in their stationary phase of growth having a reduced recovery potential no or only a slight statistically not significant inhibition of recovery by Quinacrine occurred A difference between the recovery processes in expo nentionally growing and stationary phase cells one process being more affected by Quinacrine than the other can be one explanation for the observation This explanation is supported by calculations which indicated that the extrapolation number (n) of the survival curves which can be regarded as an ex

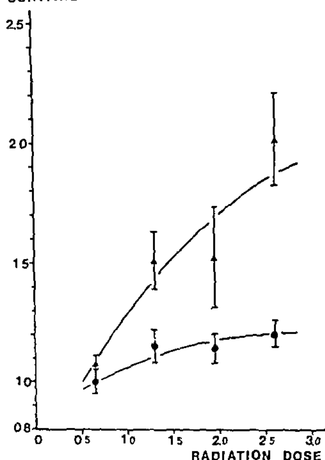


Fig 4 Survival ratio of Quinacrine untreated to Quinacrine treated cells after irradiation (Gy) within a low dose region Means ± SE *are illustrated* from replicate experiments with cells in their exponential (▲ 9 paired experiments) or stationary (● 7 paired experiments) growth *phase*

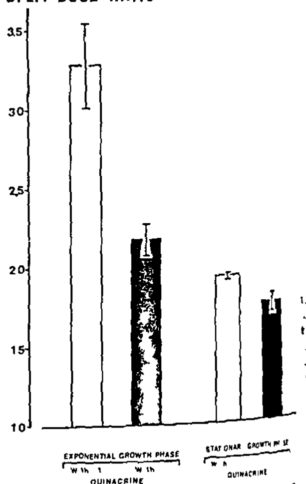


Fig 5 Split dose ratio of Quinacrine treated and Quinacrine-untreated cells after irradiation with 2×3 9 and 1×7 8 Gy Columns illustrate Means ± SE from replicate experiments with cells in their stationary (8 paired experiments) or exponential (8 paired experiments) growth phase

pression for the recovery potential of the cells in the case of *exponentially* growing cells (about 3 8) was reduced to al*most the* same value (about 2 3) as in the case of stationary phase cells *(about 2 5)* In agreement with this finding the split dose ratio for the exponentially *growing* cells is also reduced to about the same *value* as the ratio for the stationary phase cells (around 2) Alternatively the difference between the *effect of* Quinacrine on the cells in the 2 phases of *growth can* be explained also by the standard deviation of the differences in the 2 cases the significance of *small differences* for stationary phase cells be*ing more difficult* to evaluate statistically

Working with two strains of E *coli one* possessing an efficient repair system for radiation induced DNA single strand breaks *and* the other deficient of

Thus their results suggest a relationship between DNA injury and survival Such a relationship is suggested also by the present results in consideration of previous observations (MODIG et coll) made with the present Chinese hamster cell line and which indicated an inhibition of the repair of DNA single strand breaks by Quinacrine On the other hand VOICULETZ et coll argue that no relationship between Quinacrine induced DNA injury and reaction exists since they did not find any difference in the extrapolation number of the survival curves of Quinacrine treated and Quinacrine untreated Chinese hamster ovary cells This argument based upon negative observations needs further experimental support from more direct tests e g using split dose experiments or experiments in a low dose

ent of malaria and other infectious diseases. This xperience could be of a great value if Quinacrine as of any advantage in radiation therapy. At resent only few reports on the sensitizing effect of Quinacrine on experimental tumors in vivo ave appeared (ACKERMAN et coll. 1965, ALGHIL. ri 1966). It is conceivable that Quinacrine is useful the treatment of tumors which have a higher potential to recover from radiation injury than normal ssues, e.g. in malignant melanoma (DEWEY 1971). he present data suggest further investigations in us respect.

SUMMARY

The effect of Quinacrine on the recovery of cells from ublethal radiation injury was analysed by testing cellular rvival after exposure to low and high radiation doses nd after irradiation with split doses. Quinacrine was und to inhibit recovery in cell cultures in exponential owth phase but not, or only slightly, in cells in stationary owth. This finding is related to the inhibitory effect of uinacrine on the repair of radiation induced DNA strand eaks.

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CALCITONIN AND MAMMARY CARCINOMA

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Ectopic production of hormones by neoplasms is a relatively unknown subject that may offer diagnostic possibilities The calciotropic hormone calcitonin is known to be produced by the C cells of the thyroid gland The plasma calcitonin is usually increased in patients with medullary thyroid carcinoma (DEFTOS 1978) Increases in plasma calcitonin have also been found in patients with other endocrine malignancies e g oat cell carcinoma of the lung and breast carcinoma (SILVIA et coll 1974 COOMBES et coll 1976) Especially when dealing with metastatic breast carcinoma the plasma calcitonin level is reported to be increased in a majority of patients (COOMBES et coll 1976 OLSEN et coll 1978) An evaluation has been performed to elucidate whether determination of immunoreactive plasma calcitonin could be of any value in the preoperative examination of patients with breast carcinoma and whether calcitonin thus could be named a tumor marker The results are now reported

Material and Methods

The material consisted of 33 women mean age 56 8 years (range 36–88) Clinical examination scintigraphy of the liver and skeleton and microscopic examination of the breast specimen revealed no metastases in 13 patients

Ten women had regional lymph node metastases and another 10 had known bone or other metastases

Blood samples were obtained for determination of calcium phosphorus creatinine and calcitonin Calcitonin was determined in serum by the radioimmunoassay method described by ALMQVIST et coll (1974) All determinations were made twice The coefficients of variation were 18 per cent The calcitonin levels were classified as normal or elevated

Results

All patients except 3 had normal plasma calcitonin levels (Table 1) Two of the 3 patients with elevated calcitonin had axillary lymph node metastases and bone metastases Furthermore one of these two women had large metastases in the liver and in both ovaries Eight of the patients (24 per cent) had bone metastases but only 2 of these had elevated plasma calcitonin Osteolytic bone metastases were found in 5 of these 8 women but only 2 of them had elevated plasma calcitonin All 3 patients with elevated plasma calcitonin had normal serum calcium and phosphorus as well as creatinine

Discussion

Several authors (COOMBES et coll 1975 OLSEN et coll RASMUSSON et coll 1978) have found elevated plasma calcitonin in patients with breast carcinoma and particularly when distant metastases were present (Table 2) However in the present series no correlation between plasma calcitonin level and women with breast carcinoma existed even in the presence of bone metastases

The absence of correlation in the present series might be due to the fact that in the preparation of antibodies to human calcitonin an extract from medullary carcinoma of the thyroid was used

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(ALMQVIST et coll) This may produce antibodies of another aminoacid composition than the corresponding calcitonin antibodies produced by breast carcinoma tissue This suggestion is made as calcitonin from different species can differ in the aminoacid structure (DEFTOS) Such immunoheterogenicity has been further proved by ROOF et coll (1979) in a series of patients with different types of malignant disease However high molecular weight immunoreactive calcitonin has been found in the plasma of patients with medullary carcinoma of the thyroid as well as in the medias in which breast carcinoma cells or cells from medullary carcinoma of the thyroid have been cultured (COOMBES et coll 1976 DEFTOS GOLTZMAN & TISCHLER 1978) This may indicate that the calcitonin antibodies should react towards calcitonin of both types of tissues

The method used differs in one point from the method used by OLSEN et coll who found a high correlation between calcitonin and breast carcinoma They used synthetic calcitonin instead (DIETRICH et coll 1975) However this should not be responsible for the difference as mentioned

Another reason for the poor correlation may be that calcitonin is not produced in the patients until bone metastases and mainly osteolytic metastases have appeared OLSEN et coll found that plasma calcitonin was frequently elevated in patients with osteolytic metastases However in the present material 5 of 8 patients had metastases of this type but only 2 of them had elevated plasma calcitonin The elevation of calcitonin in these patients could be a secondary effect and produced by the C cells of the thyroid gland as a result of hypercalcemia due to osteolytic metastases Recently JØRGENSEN et coll (1979) reported on 137 patients with breast tumours They could not prove any increase in immunoreactive calcitonin in either benign or malignant tumors or the adjacent tissue This is further emphasized in the present series and a similar conclusion can be drawn from the results of RASMUSSON et coll

It may thus be concluded that the determination of plasma calcitonin is of no value in the evaluation of patients with breast carcinoma and it should not be referred to as a tumor marker of breast carcinoma

SUMMARY

Plasma calcitonin was determined in 33 patients with breast carcinoma Eight patients had bone metastases as revealed at scintigraphy and radiography Only 3 of the 33 patients had elevated plasma calcitonin 2 had osteolytic bone metastases It is concluded that the determination of plasma calcitonin is of no value in the evaluation of patients with breast malignancy and it should not be referred to as a tumor marker

Table 1

Calcitonin in patients with breast carcinoma with or without visible metastases

	Normal	Raised
No metastases	12	1
Regional lymph node metastases	10	0
Bone metastases	8	2
(osteolytic bone metastases)	(5)	(2)
Total	30	3

Table 2

Different series of plasma calcitonin in breast carcinoma

	Number of patients with elevated plasma calcitonin	
	With or without regional metastases	With bone metastases
COOMBES et coll (1975)	4/30	[illegible]/8
OLSEN et coll (1978)	6/21	10/13
RASMUSSON et coll (1978)	0/8	1/6
Present series	1/23	2/10

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EFFECT OF A SCREENING PROGRAMME ON BREAST CARCINOMA INCIDENCE MORTALITY AND SURVIVAL

IRMA SOINI and MATTI HAKAMA

he malignant cases detected in screening are an ufficient basis for an evaluation of the program

The incidence and mortality of the screened ulation compared with the non screened popula n provide a better indication of the efficacy of the gramme Primary results of screening for breast cinoma i e the numbers of malignant cases de ted have been amply reported but the follow up the women screened has often been incomplete d of short duration 10 years at the longest (MIL R 1978) In some programmes health education on f-examination of the breast has also been given ANGELAND 1970 DAVEY et coll 1974) but no ults on its effect on the subsequent risk of breast rcinoma have been reported

The local Cancer Society in one Finnish city or nized a breast carcinoma screening programme in 74 to 1975 with special attention paid to health ucation (SOINI & LAUSLAHTI 1976) The effect of e programme on incidence mortality and survival ll be given in the present report

Material and Methods

All women aged 41 to 60 years (i e all women rn in 1914–1933) in Tampere an industrial city in ntral Finland were invited to attend the screening ogramme during the period April 1974 to March 975 In 1974 Tampere had a population of 167 750 ncluding 20 644 females in the age group invited

In the screening the breasts and the axillary lymph odes were examined and palpated by specially ained nurses Women with abnormal findings were eferred to a physician for further examin ti n The indications for this further examination were a lump retracted nipple secretion change in the skin of the breast and the nipple big breasts difficult to palpate pain in the breasts and enlarged axillary lymph nodes The women were also instructed in monthly self-examination and given an illustrated leaflet published by the Cancer Society of Finland with further instructions

Further examinations by a physician included palpation and if indicated fine needle biopsy or secretion specimen mammary radiography and thermography A woman with abnormal findings re quiring no surgery was asked to undergo a new examination at a later date Patients who were re ferred for surgery as a result of the palpation radio-graphy and thermography or cytologic examination were operated upon at the local City Hospital

The follow up of the screened population (ad 30 6 1978) was based on the figures of the local histologic laboratories (incidence and stage of car cinoma) on the death certificate reports of the Office of the City Physician (mortality) and on the records on individuals of the group in the National Population Registry (emigration) Only women who did not move from the area were followed in 1974 to 1978 because the follow up of the total group would have caused a considerable delay in the evaluation of the results

New cases of and deaths from breast carcinoma among females aged 41 to 60 in Tampere in 1960 to 1973 were found from the original records of the Finnish Cancer Registry The Finnish Cancer Regis

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try is a population based registry which covers the entire country All hospitals pathologic and cytologic laboratories and practitioners have been requested to make reports to the Finnish Cancer Registry of all new cases of malignant tumours since 1953 The registry receives a copy of every death certificate in which mention is made of malignant disease The files of the Finnish Cancer Registry are cross checked annually against the lists of all deaths occurring in Finland

The incidence and mortality figures in this report for breast carcinoma for 1960 through 1973 were cross sectional rates by calendar period for women in Tampere aged 41 to 60 whereas the estimates of incidence and mortality for the screened population in 1974 to 1978 were rates for women born 1914 to 1933 The observed rates in 1974 to 1978 were compared with those expected from extrapolation of the linear trend in 1960 to 1973

Survival rates were calculated by the actuarial method (BERKSON & GAGE 1950) The malignant cases were divided into three groups those diagnosed in 1960 to 1963 1970 to 1973 and 1974 to 1977 according to whether they were diagnosed before during or after the screening programme The patients diagnosed in 1960 to 1963 and 1970 to 1973 were followed up to the joint closing date of 31 12 1974 through the Finnish Cancer Registry whereas the most recent group was followed up to 31 12 1977 by means of data from the Office of the City Physician Consequently the follow up periods were 12 to 15 2 to 5 and 0 to 4 years respectively Deaths from causes other than breast carcinoma were found only in the group diagnosed in 1960 to 1963 (9 cases) The deaths were treated as withdrawals alive when the survival rates were estimated In this case the method gave the same result as that based on competing risks of death (CHIANG 1968)

Results

Of the 20644 women invited to attend the screening programme 17261 (84%) actually did so The examining nurses referred 627 (3 6%) women for further examination which was performed in 615 Of these 615 117 i e 0 7 per cent of the entire series were referred to the hospital for surgery Twenty seven had malignant tumours and 90 benign lesions Four of the malignant cases were non infiltrative intraductal carcinomas and seven were preclinical

Table 1

The stage of new cases of breast carcinoma in Tampere in 1960 to 1978 Women aged 41 to 60 years in 1960 to 1973 women born 1914 to 1933 in the screened population in 1974 to 1978

Year	Total No of cases	Stage unknown		Stage known	
		Number	Per cent of all cases	Number	Per cent localized
1960	20	0	0	20	40 0
1961	24	1	4 2	23	41 5
1962	18	5	27 8	13	69
1963	17	5	29 4	12	50 0
1964	11	2	18 1	9	[illegible]
1965	14	2	14 3	12	33 3
1966	25	4	16 0	21	[illegible]
1967	15	6	40 0	9	66 7
1968	12	2	16 7	10	40 0
1969	24	2	8 3	22	31 8
1970	18	1	5 6	17	58 8
1971	28	0	0	28	46 4
1972	25	1	4 0	24	50 0
1973	27	1	3 7	26	53 8
1974	29	0	0	29	41 4
1975	28	0	0	28	60 7
1976	19	0	0	19	58 0
1977	23	0	0	23	70 0
1978	10	0	0	10	60 0

Table 2

New cases of breast carcinoma and deaths in 1960 to 1973 among women aged 41 to 60 years in Tampere

Year	Person years at risk	New cases		Deaths	
		Number	per 10^3	Number	per 10^3
1960	19 200	0	1 04	13	0 68
1961	19 330	24	1 24	14	[illegible]
1962	19 460	18	0 92	9	0 46
1963	19 590	17	0 87	8	0 41
1964	19 720	11	0 56	10	0 46
1965	19 850	14	0 71	5	0 50
1966	19 980	25	1 25	4	0 25
1967	20 110	15	0 75	7	0 20
1968	20 240	12	0 59	9	0 35
1969	20 370	24	1 19	10	0 44
1970	20 507	18	0 88	8	0 49
1971	20 531	28	1 36	10	0 39
1972	20 578	25	1 22	9	0 49
1973	20 597	27	1 31	9	0 44

Table 3

ew cases of breast carcinoma and deaths in the screened population 1974 to 1978 for women in Tampere born in 1914 to 1933

Year	Person years at risk	New cases Number	New cases per 10³	Deaths Number	Deaths per 10³
1974	20 644	29	1 40	9	0 44
		13			
		16			
1975	20 0?	28	1 39	9	0 45
		14			
		14			
1976	19 593	19	0 97	8	0 41
1977	19 305	23	1 19	5	0 26
1978	9 53?	10	1 05	2	0 21

In the screening

Outside the screening

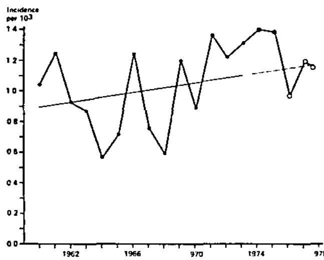


Fig 1 Observed and expected breast carcinoma incidence rates in 1960 to 1978 in Tampere Cross sectional rates in 1960 to 1973 for women aged 41 to 60 years group rates in 1974 to 1978 for women born in 1914 to 1933 During screening (●) After screening (○)

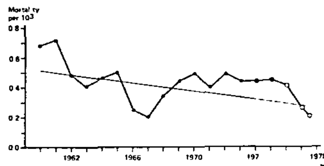


Fig 2 Observed and expected breast carcinoma mortality rates in 1960 to 1978 Cross sectional rates in 1960 to 1973 for women aged 41 to 60 years group rates in 1974 to 1978 for women born in 1914 to 1933 Residents of Tampere at the time of diagnosis During screening (●) After screening (○)

non palpable carcinomas Two cases were diagnosed among the 615 women who attended the further examinations during the follow up period 1975 to 1978 and one of them was found in the group (136 cases) referred for closer surveillance by the physician Localized carcinomas detected in the screening accounted for 55 6 per cent of the total The figure was 41 4 per cent of all diagnosed malignant tumours in 1974 and 60 7 per cent of those in 1975 The percentages of localized carcinomas year by from 1960 to 1978 are presented in Table 1

During the period 1960 to 1973 11 to 28 new breast carcinoma cases and 4 to 14 deaths from breast carcinoma were registered corresponding to the incidence of 0 6 to 1 4 per 10³ and mortality 0 2 to 0 7 per 10³ annually (Table 2)

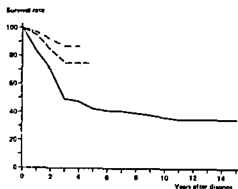
In 1974 16 and in 1975 14 further malignant cases were detected outside of the screening programme in the female population invited to participate in the programme in addition to the 27 new breast carcinoma patients diagnosed on the basis of screening Consequently the total incidence in 1974 was 1 40 per 10³ and in 1975 1 39 per 10³ The trends in incidence and mortality during and after screening are presented in Table 3 The screening was associated with a slight increase in incidence in 1974 to 1975 and a slight decrease during follow up period The mortality figures decreased during the total period after screening

The expected incidence and mortality figures in 1974 to 1978 were calculated from the linear trend in 1960 to 1973 A comparison of the observed figures with those expected reveals no substantial change during and after the screening programme (Figs 1 2)

The survival curves of the patients with carcinoma diagnosed in 1960 to 1963 1970 to 1973 and 1974 to 1977 are presented in Fig 3 The rates increased by the year of diagnosis and the difference in 4 year rates between patients diagnosed in 1960 to 1963 and 1974 to 1977 was 40 per cent

Discussion

After effective screening the incidence of the disease should be lower during the next few years than during the actual screening period The successful



)bserved survival curves (in per cent) of breast carcinoma ts n 960 to 1963 (—) 1970 to 1973 (women aged 41 to 60 (ı (---) and 1974 to 1977 (all women born in 1914 to 1933) Residents of Tampere at the time of diagnosis

therapy of early malignant cases detected in the screening should cause a decrease in mortality and an increase in survival An early diagnosis should also result in an increased proportion of localized cases The incidence of and mortality from breast carcinoma are the most reliable indices for evaluating the efficacy of a screening programme There is evidence that screening for breast cancer decreases breast cancer mortality (MILLER 1978)

Because the screening programme should detect preclinical early cases the incidence of new cases in the screening consists of normal incidence during the screening period and of the prevalence of preclinical malignant cases in the screened population The number of preclinical cases (7/27) in the present screening programme was small This result is likely to be due to the primary screening method which was only palpation A preclinical tumour is non palpable by definition Consequently only some of the preclinical cases were likely to be detected in the screening

A high risk group of 627 females from the screened population of 17261 women was identified in the clinical examination Besides tumours confirmed in the screening only 2 malignant cases were detected during the follow up period in this group Accordingly the screening was also an inadequate method of defining a high risk group This is in agreement with the result that benign lesions are independent of the risk of breast malignancy (SOINI & HAKAMA 1979)

The increase in the incidence during screening as well as the decrease after screening were almost non-existent (Tables 2 3) The deviations of incidence and mortality from the expected figures estimated on the basis of the trend in previous years (1960 to 1973) were also insignificant (Figs 1 2) The improvement in survival rates by calendar period has continued and there was no evidence that the survival rates of the patients diagnosed during screening were better than expected on the basis of long term trends (Fig 3) Further thanks to screening early detection apparently lengthens the survival time through the length bias (ZELEN 1976) and survival rates do not provide a reliable basis for the evaluation of the screening

The proportion of localized carcinomas in the screened population should be higher than in the non screened one In this screening the proportion of localized carcinomas was higher than in the previous years but did not deviate from the general trend The fact that the proportion of localized tumours had generally increased can be accounted for by change in the biologic type of the disease a change in diagnostic criteria health education or the combined effect of all these factors

All immediate indices of the efficacy of the screening higher frequencies of localized cases improved survival decreases in incidence and mortality when the screening period (1974 to 1975) was compared with the subsequent years (1976 to 1978) would indicate that screening was beneficial However consideration of longer trends raised serious doubts as to any major effect the screening programme might have on the risk of malignant tumours of the breast The results can be accounted for by factors other than the screening Changes in incidence mortality survival and the proportion of localized cases essentially followed the expected long term trend Furthermore the improved survival rates were affected by the length bias and did not give any reliable basis for an evaluation of the screening The present results emphasize the need for a properly defined control group Proper controls are found if the screening programmes follow an experimental design when the effects of screening on the risk of disease are evaluated

SUMMARY

Screening for breast carcinoma revealed 27 cases of malignancy 7 of them preclinical *The method consisted of palpation and health education Mammary radiography and fine needle biopsy were used in uncertain cases* Shortly after the screening the incidence and mortality were lower (1 07 and 0 29 per 10³ respectively) than during the screening (1 40 and 0 45 per 10³) Survival and the percentage of localized cases was

patients diagnosed during or after screening than for atients diagnosed before However the changes did not how any substantial differences from the long term ends It was concluded that the screening programme no real effect on the malignancy risk The absence of ffect was accounted for by the relatively inefficient ing method

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FROM NORSK HYDRO S INSTITUTE FOR CANCER RESEARCH THE NORWEGIAN RADIUM HOSPITAL AND THE NORWEGIAN CANCER SOCIETY MONTEBELLO OSLO NORWAY

EFFECT OF SINGLE DOSE IRRADIATION ON THE PROLIFERATION KINETICS IN A HUMAN MALIGNANT MELANOMA IN ATHYMIC NUDE MICE

E K ROFSTAD T LINDMO and T BRUSTAD

Design of optimum fractionation regimes in radiation therapy requires among other factors detailed information on the kinetics of cell death and cell proliferation after each fraction The proliferation kinetics in a considerable number of non irradiated animal and human tumours and normal tissues have been reported (for review cf STEEL 1977) but the effect of irradiation on the proliferation kinetics has been analysed only for a few types of tissue However partial cell synchrony shortly after irradiation due to mitotic delay or selective inactivation of cells in the most sensitive phases of the cell cycle has been demonstrated for both tumours and normal tissues (KALLMAN et coll 1966 WITHERS & ELKIND 1969 DENEKAMP et coll 1969 ROCKWELL & KALLMAN 1974) The cell cycle time of cells of normal tissues has been shown to be shortened during regrowth (CAIRNIE 1967 LESHER 1967 BROWN & BERRY 1969 BROWN 1970) but results from tumours during regrowth are conflicting Both shortened prolonged and unchanged cell cycle times as well as increased and reduced growth fractions have been reported (TUBIANA et coll 1968 TUBIANA 1971 BROWN & BERRY BROWN HERMENS & BARENDSEN 1969 1970 1975 FRINDEL et coll 1970 DENEKAMP & THOMLINSON 1971 VAN PEPERZEEL 1972 SZCZEPANSKI & TROTT 1975 KOVACS et coll 1976 NELSON et coll 1976 BRAUNSCHWEIGER et coll 1979) Further investigations of these problems seem to be needed particularly of experimental tumours which may be representative models for human tumours Thus the cells of human tumours grown in immune deficient animals have been shown to have phase durations in the range of those found for cells of human tumours in man (PICKARD et coll 1975 ROFSTAD et coll 1977a) Therefore the effect of single dose irradiation on the proliferation kinetics in a human malignant melanoma grown in the athymic mutant nude mouse was analysed The results are discussed in relation to the radiation response of the tumour as determined from measurements of single cell survival and tumour size

Materials and Methods

Young male nu/nu/BALB/c/BOM mice were used The animals were kept under conventional conditions in a special room at 27°C with automatically regulated 12 hour periods of light and darkness

The malignant melanoma (E E malignant melanoma) was taken from a lymph node metastasis in the left axilla of a 62 year-old man The tumour tissue was composed of melanin poor atypical nevus cells growing in large balls Cells and nuclei varied greatly in size and shape Innumerable mitoses were seen

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Passages 16–22 were used The histologic appearance of the tumour had not changed after 22 passages in nude mice Calipers were used to measure the length of two perpendicular axes of the tumours and the cross sectional area of the tumours calculated as being elliptical was used as a parameter for tumour size The cross sectional area of the tumours ranged from 50 to 100 mm² on the day the experiments were initiated

Irradiation procedure The mice were irradiated locally without anaesthesia at a focus skin distance of 80 cm by applying one tangential radiation field from a 5000 Ci (1 85×10¹⁴ Bq) ⁶⁰Co therapy unit (AEM Mobaltron 80) Acceptable dosimetric conditions were obtained by immersing the mice partly in a water phantom whereby the tumours were positioned under water and at a distance from the outer surface of the phantom wall greater than that corresponding to the ionization maximum of the radiation used Perspex tubes served as mouse holders The water temperature was kept at 30°C Measurements made with thermoluminescent discs (TLD 100 LiF Ribbon Harshaw) placed at the ionization maximum varied within a few per cent of the corresponding ionization chamber readings of 1 4 Gy/min Further details of the irradiation procedure are described elsewhere (ROFSTAD et coll 1977b)

Flow cytofluorometry The tumours were finely minced in phosphate buffered saline by means of a scalpel and a pair of tweezers and the tissue suspensions were filtered through 100 μm filters (Nytal Schweizerische Seidengazefabrik AG) Cell debris and injured cells were separated from the intact cells in a filtered cell suspension by a centrifugation technique based on the same principles as those described by SEGLEN (1976) for rat liver cells The cell suspension was layered over a 15 per cent metrizamide cushion and after centrifugation at 1 000 rpm for 5 min most of the injured cells were sedimented to the bottom of the tube while the intact cells were layered at the interface The supernatant containing cell debris was removed and after a second centrifugation at 2 000 rpm for 5 min a clean cell suspension was obtained The cells were stained with mithramycin without previous fixation (CRISSMAN & TOBEY 1974) and analysed by flow cytofluorometry The 457 nm line of a 4 W argon laser was used for excitation of the mithramycin fluorescence which was detected at wavelengths longer than 476 nm DNA histograms were registered in 128 channels of the multichannel analyzer o the flow cytofluorometer (LINDMO & STEEN 197 LINDMO & PETTERSEN 1979)

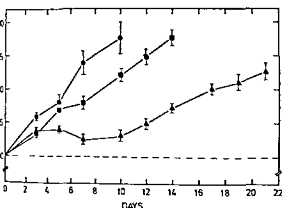
In vitro colony assay A soft agar colony assa based on the technique described by COUR TENAY (1976) and COURTENAY & MILLS (1978) wa adapted for E E malignant melanoma The cultur medium used was Ham s F12 medium with 20 foetal calf serum 250 mg/l penicillin and 50 mg streptomycin (Gibco Biocult)

Single cell suspensions were prepared from th tumours without the use of enzymes The tumour were minced mechanically in culture medium an the tissue suspensions were filtered through 30 μ filters (Nytal Schweizerische Seidengazefabri AG) The cell concentration was determined by us of a hemocytometer viewed through a microscop with phase contrast optics Intact cells which did no take up trypan blue were counted as viable Th cell suspensions were diluted to appropriate con centrations in culture medium

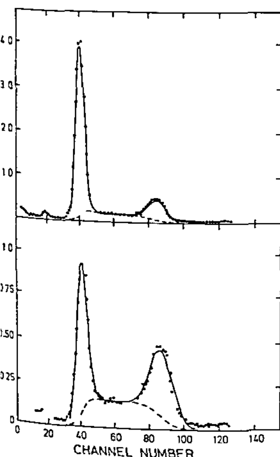
Suspensions of red blood cells (RBC) were pre pared from blood of hooded/NOR rats The seru and the buffy coat were removed after centrifuga tion The RBC were rinsed in phosphate buffere saline three times before final resuspension in cul ture medium to the original blood volume Th RBC suspensions were stored up to three weeks a 4 C Immediately before use they were diluted 1 in culture medium and heated to 44 C for 1 h t destroy remaining nucleated cells

Agar solutions were prepared by boiling pow dered agar (Bacto agar Difco) with bidistilled wa (0 05 g/ml) for 10 min After being autoclaved a 120 C for 15 min the 5% agar solutions were coole to 44°C in a water bath and mixed with cultur medium at 44 C to make agar solutions of 0 5 pe cent The final soft agar was made by mixing volumes of 0 5% agar solution 3 volumes of tumou cell suspension and 1 volume of RBC suspensio The temperature of the latter two cell suspensio was 37°C before they were added to the agar so ution which had been moved from the water-bath a 44°C to the incubator room at 37°C a few minute earlier

Aliquots of 1 ml were immediately seeded in gla tubes Tubes with ground glass joints and corre pondingly coned ground glass stoppers were use Stopcock grease was applied in the joints to ma the tubes gastight The tubes were plunged i crushed ice after seeding and the soft agar wa



1 Mean relative tumour cross-sectional area as a function of after single dose irradiation of a human malignant melanoma n subcutaneously in nude mice Vertical bars indicate SD of mean ● Non irradiated tumours (n=6) ■ Tumours ir ted with 5 0 Gy (n=8) ▲ Tumours irradiated with 10 0 Gy 8)



? DNA histograms analysed by fitting a mathematical mod —) to the experimental data (●) Dashed curves show the lel s estimate for cells in S The distribution (in per cent) of s among the various phases—G /G 65 S 20 and G +M 15 in -irradiated tumour (top) and G /G 34 S 35 and G_2+M 31 in our 4 h after exposure to a single dose of 0 0 Gy (bottom)— obtained from the mathematical analysis The peaks around nnel 19 are due to non parenchymal mouse c l The channel nber is proportional to cellular DNA content

allowed to set Immediately afterwards the tubes were flushed with a gas mixture of 5% O 5% CO and 90% N The flushing was repeated about 2 h later The tubes were incubated at 37°C in vertical position for 3 weeks Culture medium (2 ml) was added 5 days after seeding and changed 8 days later The tubes were flushed on each occasion and in addition 3 9 and 17 days after seeding

Colonies larger than 40 cells were counted 21 days after seeding The agar lumps were decanted and carefully squeezed out with a glass-cover on to a specially made sectioned glass plate with a 2 mm high rim The agar sheets were analysed through a stereo microscope

Autoradiography The mice were injected in traperitoneally with a single dose of 1×10^{-6} Ci/g ($3\ 7 \times 10^{4}$ Bq/g) bodyweight (methyl ^{3}H) thy midine (18 8 Ci/mmol $6\ 96 \times 10^{11}$ Bq/mmol) at 8 a m At predetermined times the mice were killed and the tumours fixed in 4 per cent formaldehyde The tumours were then embedded in paraffin wax and sections 3 to 4 μm thick were cut and mounted on slides The tissue sections were coated with Il ford L4 nuclear research emulsion by the dipping technique After an exposure time of 8 weeks at 4°C the slides were developed in Kodak microdol X fixed in Kodafix and stained with hematoxylin and eosin The mean grain counts over labelled inter phase nuclei ranged from 10 to 15 The average background corresponded to less than one grain per cell nucleus and a labelling criterion of minimum three grains per nucleus was adopted In order to determine the percentage labelled mitoses (PLM) curves 300 mitotic figures from each of the tumours were scored as labelled or not The labelling indices were determined by analysing 10000 cells from tumours of mice killed 2 h after the thymidine injec tion

The PLM-data were analysed by assuming that the time spent by the cells in each of the phases of the cell cycle is described by independent log nor mal distributions (BARRETT 1966) Theoretical PLM-curves were calculated from different chosen sets of distributions by applying a Monte Carlo program (ROFSTAD et coll 1977a) The set of dis tributions which gave the best fit to the experimental data was selected by visual inspection

Results

The mean tumour cross sectional area as a func tion of time after exposure to single radiation doses

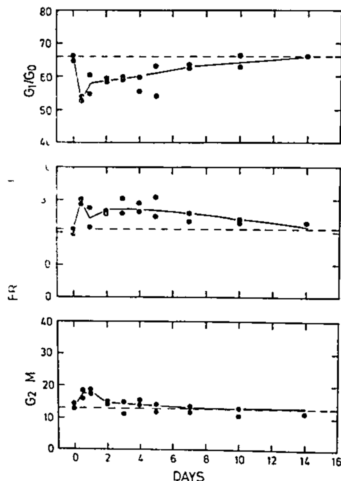


Fig 3 Fracti n f e l ı per cent) in G_1/G S and G_2+M as a fun t on of me ı ter xpo ure to a single dose of 5 0 Gy for a human mal nai mel noma grown in nude mice Values deter min d fr m ar lys s t DNA histograms The dashed lines repre sent the al e in n irradiated tumours

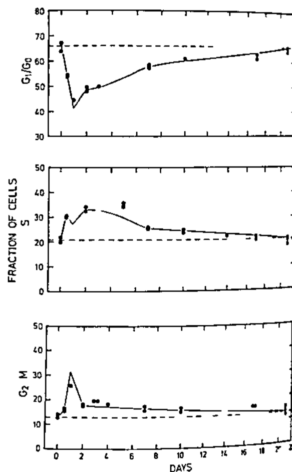


Fig 4 Fraction of cells (in per cent) in G/G S and G_2+M as a function of time after exposure to a single dose of 10 0 Gy for a human malignant melanoma grown in nude mice Values determined from analysis of DNA histograms The dashed lines represent the values in non irradiated tumours

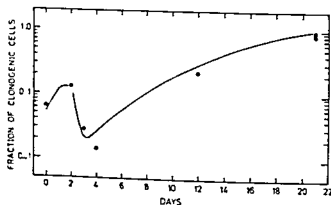


Fig 5 Fraction of clonogenic cells in a human malignant melanoma grown in nude mice as a function of time after exposure to a single dose of 10 0 Gy Each point represents one tumour Values calculated in relation to the plating efficiency of cells of untreated tumours of the same size

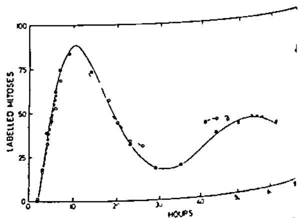


Fig 6 Percentage labelled mitoses curves for a human malignant melanoma grown in nude mice Non irradiated tumours (●) Tumours dissected free 8 to 11 days after exposure to a single dose of 10 0 Gy (O)

Table 1

arameters defining the log normal distributions describing the hase durations of the cells of E E malignant melanoma grown in nude mice

hase	Controls tumours			Irradiated tumours		
	Median	Mean	SD	Median	Mean	SD
ı	19 0	?1 4	11 0	1? 7	14 9	9 1
	13 3	15 1	8 1	1? 5	15 3	10 8
₋	5 0	5 7	3 0	5 3	6 1	3 5
I	1 0	1 0	0 3	1 0	1 0	0 3
	41 1	43 ?	14 0	34 7	37 3	14 6

^{3}H Tdr was administered to the mice 8 days after the tumours rere exposed to a single radiation dose of 10 0 Gy

The distribution for the total cell cycle time T is assumed to e a log normal distribution with mean $m = \Sigma\ m$ and SD $=\sqrt{\Sigma\ _i\sigma^2}$ where m_i and σ_i are the mean and the SD of e four phases into which the cell cycle is divided The median M of this distribution is

$$M = \frac{m}{\sqrt{1 + \frac{\sigma}{m}}}$$

of 5 0 and 10 0 Gy appears in Fig 1 The tumour ross sectional areas immediately before irradiation were normalized to unity The tumour growth was only slightly delayed after 5 0 Gy The mean tumour ross sectional area and hence the mean tumour volume increased beyond day 8 after exposure to 10 0 Gy but the increase was not as fast as for non irradiated tumours of the same size

A DNA histogram representative for non irradiated tumours is illustrated in Fig 2 (upper part) The fraction of the cells in G_1/G_0 S and G_2+M as indicated in the figure legend was calculated by assuming normal distributions for the G_1/G_0 and the (G +M)-peaks (DEAN & JETT 1974) while the S fraction (the area under the dashed curve) was modelled as a Gauss broadened exponential function (LINDMO & AARNÆS 1979) From the analyses of the DNA histograms of 6 different non irradiated tumours the mean fraction of cells in G_1/G_0 S and G_2+M was found to be 66±2% 21±2% and 13±2% respectively Fig 2 (lower part) shows an example of a DNA histogram of an irradiated tumour (?0 0 Gy) where the cell cycle distribution is highly perturbed The fraction of cells in G_1/G_0 S and G_2+M is 34% 35% and 31% respectively The fraction of doublets in cell suspensions from both non irradiated and irradiated tumours was determined to be 5 to 8 per cent by counting the number of singlets and doublets within a defined area of a hemocytometer by use of a fluorescence microscope Thus the fraction of cells in G +M is somewhat overestimated and the fraction of cells in G_1/G_0 and S correspondingly underestimated in the present analysis

The distribution of cells among G_1/G_0 S and G +M was determined as a function of time after exposure to single doses of 5 0 (Fig 3) and 10 0 Gy (Fig 4) The G_1/G_0 fraction decreased considerably during the first 12 h after 5 0 Gy During the following days the G_1/G_0 fraction increased slowly and reached the level in non irradiated control tumours about 2 weeks after exposure Both the S and the (G_2+M) fractions have a maximum during the first 2 days after irradiation followed by a slow decrease corresponding to the increase in the G_1/G_0 fraction Similar curves were obtained after 10 0 Gy (Fig 4) However the perturbations were more marked and about 3 weeks elapsed until control levels were reached

In order to facilitate the evaluation of the flow cytofluorometric measurements more detailed analysis of the proliferation kinetics in tumours exposed to 10 0 Gy was performed Fraction of clonogenic cells as a function of time after irradiation appears in Fig 5 Each point represents the value for one tumour calculated in relation to the plating efficiency of cells from non irradiated control tumours of the same size The plating efficiency was 20 to 30 per cent Immediately after irradiation the fraction of clonogenic cells was about 0 05 The control level was not reached until 18 to 21 days after irradiation

The phase durations of the cells of non irradiated tumours and tumours exposed to 10 0 Gy were determined by the PLM technique ^{3}H Tdr was administered to mice with irradiated tumours 8 days after irradiation At this time the tumours were regrowing actively as indicated in Figs 1 and 5 The PLM curves are presented in Fig 6 The parameters defining the corresponding log normal distributions are summarized in Table 1 The labelling indices were found to be 0 190 for non irradiated tumours and 0 273 for irradiated tumours By use of the mathematics described by STEEL (1968) growth fraction potential doubling time and cell loss factor were calculated from the volume doubling time the median phase durations and the labelling indices (Table 2) It may be concluded that the durations of

Table 2

Cell kinetic parameters for E E malignant melanoma grown in nude mice

	Measured values							Calculated values		
	T_D (days)	Median duration (h)					L I	T_{pot} (h)	G F	ϕ
		G	S	G_2	M	T				
Control tumours	7	19 0	13 3	5 0	1 0	41 1	0 190	56	0 66	0 67
Irradiated tumours	14	12 7	12 5	5 3	1 0	34 7	0 273	39	0 85	0 83

T_D = volume doubling time of tumours of the present size determined from growth curves L I = labelling index T_{pot} = potential doubling time G F = growth fraction ϕ = cell loss factor T_{pot} G F and ϕ were calculated from the following equations

$$\ln(1+G\ F) = \frac{T\ \ln 2}{T_{pot}} \quad L\ I = \exp\left[\frac{\ln 2}{T_{pot}}(T_G + T)\right] - \exp\left[\frac{\ln 2}{T_{pot}} T_G\right] \quad \phi = 1 - \frac{T_{pot}}{T_D}$$

(Steel 1968)

^{3}H Tdr was administered to the mice 8 days after the tumours were exposed to a single radiation dose of 10 0 Gy

G_2 and S are not significantly different in irradiated and non irradiated tumou while G_1 is shortened in irradiated tumours The growth fraction and the cell loss factor are considerably larger in irradiated tumours than in non irradiated tumours

Discussion

A previou investigation has indicated that the phase durations of the cells of human tumours grown in nude mice are in the range of those found for tumour cells in man (ROFSTAD et coll 1977 a) The phase durations of the cells of non irradiated E E malignant melanoma are in agreement with this conclusion

The labelling index of non irradiated tumours (0 190) indicates that all cells in the S fraction as measured by flow cytofluorometry (0 21) are synthesizing DNA i e it is not likely that a significant proportion of the cells are arrested in S As the growth fraction was found to be 0 66 (66%) and the (G +M) fraction contributes about 13 per cent of the population most of the non proliferating cells (34%) must have the same DNA content as proliferating cells in G_1 A corresponding analysis of the data obtained for irradiated tumours (Table 2 Fig 4) leads to the same conclusion Similar results were previously obtained for untreated NHIK 1922 cells grown in nude mice (ROFSTAD et coll 1980)

Figs 2 3 and 4 show that tumour cells are accumulated in G +M during the first day after irradiation This accumulation is to be expected since radiation induced mitotic delay is well known from considerable number of investigations both in vit and of solid tumours By assuming that the flux cells to G_1/G_0 is negligible during the first 24 h af the tumours are exposed to a high radiation dos and since the median duration of G_1 in non- radiated tumours is 19 0 h it follows that G₁/G₀ peak of the DNA histograms observed 24 after the exposure may be ascribed mainly to no proliferating cells or slowly proliferating G_1-cel The fraction of cells in the G_1/G_0 peak 24 h af exposure to 10 0 (ca 40% Fig 4) and 20 0 Gy (c 35% Fig 2) corresponds fairly well with the fra tion of non proliferating cells in non irradiat tumours (34%) Thus also the DNA histograms o tained 24 h after exposure to 10 0 and 20 0 Gy ind cate that most of the non proliferating cells in t tumour have the same DNA content as proliferat cells in G_1

The DNA histograms obtained with the prese technique include radiation inactivated cells wh are morphologically intact The fraction clonogenic cells was about 5 per cent immedia after exposure to 10 0 Gy and not until 4 days la did this fraction start to increase towards the val in non irradiated tumours (Fig 5) The DNA his grams from the first day after irradiation were t mainly based on cells lethally damaged by radiat The radiation inactivated cells became less impo tant with time after irradiation as they disintegra and were removed

The present experiments may explain some of th

mechanisms underlying the gross radiation response of E E malignant melanoma Even though about 5 per cent of the cells were inactivated after 10 0 Gy (Fig 5) the tumour volume after irradiation never decreased below its value at irradiation (Fig 1) The tumour volume continued to increase for about 4 days after exposure indicating that inactivated cells may have the ability to divide a few times before being lysed As the S and (G_2+M) fractions increased and the G_1/G_0 fraction decreased (Fig 4) the average volume per cell increased In addition the tumour volume may have increased because of radiation induced edema The following 4-day period was characterized by a moderate tumour shrinkage indicating that removal of inactivated cells now dominated Beyond day 8 after irradiation the rate of production of new cells was higher than the rate of removal of inactivated cells resulting in an increase in tumour volume

The fraction of clonogenic cells increased by a factor of 2 to 3 during the first day after exposure to 10 0 Gy (Fig 5) As the cell cycle time in this period was especially long due to mitotic delay this increase was probably not due to proliferation of surviving cells Neither is destruction of inactivated cells a probable explanation since the tumour volume continued to increase after irradiation The effect appears rather to be a consequence of repair of potentially lethal damage (HAHN et coll 1974 SHIPLEY et coll 1975) From day 2 to day 3 to 4 after irradiation the fraction of clonogenic cells decreased considerably There is evidence that cell death due to anoxia nutrient deficiency or both conditions occurs in poorly vascularized tumours (THOMLINSON 1960 TANNOCK 1972 YAMAURA & MATSUZAWA 1979) One possible explanation for the present decrease may be that a large fraction (80-90 %) of the surviving radiation resistant hypoxic cells were inactivated 3 to 4 days after irradiation not because of radiation injury to the cells themselves but rather because of anoxia nutrient deficiency or both Corresponding curves for the rat rhabdomyosarcoma R 1 (HERMENS & BARENDSEN 1969) and the rat fibrosarcoma RIB_5C (MCNALLY 1973) did not show a fall similar to that observed here It may be speculated that for tumours with shorter cell cycle time than the present one the effects of repair of potentially lethal damage and inactivation due to anoxia nutrient deficiency or both may cancel each other However when the central part of the rhabdomyosarcoma R 1 which contains mainly radiation resistant hypoxic cells was analysed separately the fraction of clonogenic cells was found to be significantly lower 2 days after irradiation as compared to immediately afterwards (HERMENS & BARENDSEN 1975) The fraction of clonogenic cells in E E malignant melanoma increased beyond the fourth day after irradiation (Fig 5) This may be due to both proliferation of surviving cells and destruction and removal of inactivated cells As the tumour volume decreased 4 to 8 days after irradiation (Fig 1) the last factor was probably the most important one in this time interval while the first factor probably dominated beyond day 8 when the tumour volume increased

The PLM investigation (Fig 6) was carried out 8 to 11 days after irradiation Figs 1 and 5 show that the tumours were regrowing actively at the time The cell cycle time in irradiated tumours was shorter than that in non irradiated tumours due to a shorter G_1 In agreement with the present results the cell cycle time in the rat rhabdomyosarcoma R 1 4 to 14 days after exposure to 20 0 Gy was found to be considerably shortened mainly because of disappearance of G_1 (HERMENS & BARENDSEN 1969 1970 1975) VAN PEPERZEEL observed a reduced duration of all phases of the cell cycle 45 h after pulmonary metastases of the murine adenocarcinoma M 8013 were exposed to 2 1 Gy On the other hand a slight prolongation of G_1 was observed in a squamous cell carcinoma of the hamster cheek pouch within 4 days after exposure to 5 0 and 10 0 Gy (BROWN & BERRY BROWN) Similarly most of the PLM-curves obtained for the murine adenocarcinoma 284 in the interval 1 to 8 days after doses of 6 0 and 12 0 Gy indicated a prolongation of the cell cycle time mainly G_1 (SZCZEPANSKI & TROTT)

In addition to reduced duration of G_1 the growth fraction in E E malignant melanoma was increased after irradiation (Table 2) Increased growth fraction was also observed in the adenocarcinomas M 8013 (VAN PEPERZEEL) and 284 (SZCZEPANSKI & TROTT) and the fibrosarcoma NCTC 2472 (MALAISE & TUBIANA 1966 TUBIANA et coll) while the growth fraction in the rhabdomyosarcoma R 1 was slightly reduced (HERMENS & BARENDSEN 1969 1970 1975)

As the cell cycle time was reduced and the growth fraction increased in E E malignant melanoma it may be concluded that the rate at which new cells were produced was higher in irradiated tumours during regrowth than in non irradiated tumours of

the same size In spite of this the volume of the irradiated tumours increased more slowly than that of the non irradiated tumours (Fig 1) due to more extensive cell loss (Table 2) Similar regrowth curves have been reported for instance for the fibrosarcoma RIB_5 (THOMLINSON & CRADDOCK 1967) and the rhabdomyosarcoma R 1 (BARENDSEN & BROERSE 1969) but also increased overall growth rate after irradiation has been demonstrated (VAN PEPERZEEL) The latter author reported the cell loss factor to be reduced after irradiation

As the majority of the non proliferating cells have the same DNA content as proliferating cells in G_1 reduced duration of G_1 and increased growth fraction should result in reduced G_1/G_0 fraction and increased S and (G +M) fractions The flow cytofluorometric measurements in Fig 4 are consistent with the ^3H Tdr incorporation data in Table 2 Thus Fig 4 indicates that the combined effect of a reduced G_1 and an increased growth fraction and hence the rate at which new cells were produced was largest during the first days after the cells were released from the G_2 block The G_1/G_0 fraction increased and the S and (G +M) fractions decreased over the next days and about 3 weeks after exposure the distribution of cells among G_1/G_0 S and G +M was the same as in non irradiated tumours Even 5 0 Gy a dose which caused minor changes in the overall tumour growth (Fig 1) affected the proliferation kinetics in the same manner as the higher dose of 10 0 Gy However the effect was less marked and ceased about 2 weeks after exposure The present results are supported by those reported by DENEKAMP & THOMLINSON In 4 animal tumours but at times corresponding to at least 10 cell cycles after doses of 10 0 and 15 0 Gy they did not observe significant changes neither in the phase durations nor in the growth fractions

SUMMARY

The effect of single dose irradiation on the proliferation kinetics in a human malignant melanoma grown in the athymic mutant nude mouse was analysed DNA histograms were obtained with flow cytofluorometry Percentage labelled mitoses curves were established by the use of conventional autoradiographic techniques Changes in the fraction of clonogenic cells with time after irradiation were measured in vitro in soft agar In non irradiated tumours the fraction of cells in G_1/G_0 S and G_2+M was 66 21 and 13 per cent respectively The median duration of G_1 S G_2 M and T was 19 0 h 13 3 h 5 0 h 1 0 h and 41 1 h respectively The growth fraction was calculated as 0 66 and the cell loss factor as 0 67 The growth fracti... increased after irradiation and the cell cycle time reduc... due to a shortening of G_1 These effects were dose depend... ent and decreased with time after exposure but were s... present after the tumours had resumed a continu... volume growth The rate of volume growth was slo... for irradiated tumours than for non irradiated tumours the same size due to a larger cell loss factor for the former

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DOSE EFFECT RELATIONSHIPS IN CERVICAL AND THORACIC RADIATION MYELOPATHIES

B HOLDORFF

In 1968 ZEMAN reported that the latency period for crosses following a single dose of irradiation was rictly time dependent Since then this question has en repeatedly discussed in connection with radia on myelopathies in man Current experience im lies that no clear negative relationship between the ose and latency period after fractionated radiation usts (FRANKE 1973) This also holds true for ani ial experiments in which a latency period of four to ve months cannot be further reduced under frac onated radiation (VAN DER KOGEL & BARENDSEN 974)

On the other hand radiation myelopathies have een reported that prove fatal after a short latency eriod and a progressive course versus other condi ions with a long interval and a long survival time hese suggest that the latency period and the extent f the lesions i e the survival time may depend pon the irradiation doses (latency period—survival ime—relationship) Thus HUNG (1968) found an verage latency period of 18 6 months among the ethal cases of his series versus an average of 25 4 onths latency in contrast to ATKINS & TRETTER 1966) as well as JELLINGER & STURM (1971) who ailed to detect any correlation between the latency eriod the severity of clinical symptoms and signs nd survival time or any dependence of these factors n the radiation dose This in turn does not corrobo- ate the result of GANSHIRT (1978) In a relatively homogeneous group of patients with complications subsequent to radiation therapy of the lymphatic system he found a rough relationship between the dose and the severity of neurologic syndromes Evi dently the question of dose effect relationships re mains a matter of considerable controversy

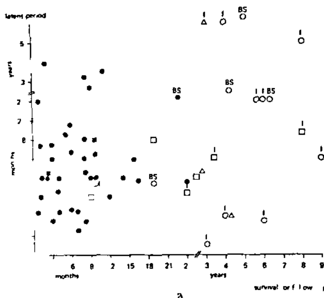
Material and Methods

The present series and well-documented cases in the literature were entered into a latency catamnesis diagram to describe the course types (Figure) and classified according to cervical and thoracic radia tion myelopathies Lumbar or thoracolumbar myelopathies were excluded from the series because they are rare and often incorrectly classified as to the exact localisation the so-called amyotrophic type being not a myelopathy at all but rather a peripheral neuropathy (HOLDORFF 1979) Only those cases in the literature were considered that pro- vided a precise and extensive documentation of the neurologic status and the course of the patients

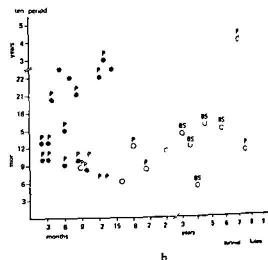
The cases of radiation therapy of the lymphatic system and myelopathies following two radiation cycles were treated separately The observation af ter irradiation of the lymph nodes was classified together with the cervical radiation myelopathies as the classification was based on neurologic findings autopsy results not always being available Occa sionally thoracically located spinal necroses could not be entirely ruled out

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Radiation myelopathies a) Cervical b) Thoracic Living (O) deceased (●) Living (□) deceased (■) after 2 series of irradiation Living (Δ) deceased (▲) after mantle field irradiation



Paraplegia (P) Brown Séquard (BS) Incomplete transverse syndrome (I)

Due to the large field of irradiation the question of the exact location of the necrosis remains open The neurologic status indicates a level which does not necessarily correspond to the level of the spinal necroses These may at times extend higher into the cervical region even though neurologic signs indicate the thoracic region of the spinal cord (CASTAIGNE et coll 1970)

These difficulties of the localization do not as a rule occur in those radiation myelopathies following a small irradiated volume because in these cases irradiation field and spinal cord lesions coincide

The appearance of the first neurologic symptoms of radiation myelopathy marked the beginning of the survival time or follow up period The terminal stages of the neurologic disease were designated by letters this was not possible in cases of cervical radiation myelopathies with a fatal outcome for which frequently no documentation of the neurologic findings at the terminal stage was available but only a status taken days or weeks before the final phase One can only speculate that in the final phase transverse syndromes with partial involvement of the upper extremities developed into upper tetraplegic transverse syndromes Paraplegias in cases of thoracic radiation myelopathies were on the other hand documented reliably

Though the relationship between the latency period and the survival or follow up period and in turn their dependence upon the radiation dose were of primary interest The extent to which various neurologic criteria and the segmental localization of the spinal cord lesion influenced course and prognosis was also analyzed

Results

The Figure does not allow a generalization and postulation of a linear relationship between the latency period and the survival time though such a relationship might be considered for some of the thoracic paraplegia cases On the other hand it is crucial for the clinical course whether the radiation myelopathy progresses to a complete transverse syndrome i e tetraplegia due to cervical lesions and to paraplegia because of thoracic ones The fatal outcome results in the majority of such cases within 10 to 18 months after the appearance of the first symptoms (black symbols in the Figure) The comparison of the latency periods of those patients who died early with those who died later—after 18 months—and with the survivors yields no statistically significant difference in the Wilcoxon test This applies to both the cervical and the thoracic radiation myelopathies Only those cases were considered in which the possible cause of death was radiation myelopathy or a secondary effect such as respiratory insufficiency with pneumonia hypostasis or pulmonary embolism The surviving patients present mainly with incomplete spinal syndromes either as Brown Séquard syndromes or as incomplete transverse syndromes these allowing a ...

Table 1

Nominal standard dose in cervical radiation myelopathies

Fatal course			Non fatal course		
NSD	Authors	Case No	NSD	Authors	Case No
14 ?	Seitz & Kalm (1961)	3	11 3	Zeman & Shidnia (1976)	
14 ?	Marty & Minckler (1973)		11 3	Jellinger & Sturm (1971)	10
14 3	Jellinger & Sturm (1971)	12	12 9	Glanzman et coll (1976)	5
14 3	Palmer (197)	7	x=14 6	Jellinger & Sturm (1971)	7
14 8	Palmer (197?)	2			
17 ?	Jellinger & Sturm (1971)	3	15 5	Solheim (1971)	3
18 1	Lechevalier et coll (1973)	5	17 3	Solheim (1971)	4
x=18 1	Lechevalier et coll (1973)	1	?0 8	Solheim (1971)	2
			Median 14 6		
18 6	Jellinger & Sturm (1971)	8			
19 1	Jellinger Sturm (1971)	4			
19 8	Holdorff (1979)	1			
0 1	Jellinger & Sturm (1971)	9			
?1 9	Jellinger & Sturm (1971)	2			
2? 5	Jellinger Sturm (1971)	11			
23 8	Lechevalier et coll (1973)	2			
Median 18 1					

vorable prognosis quoad vitam Yet both short and ong latency periods occur and the series allows no orrelation between the latency period and dose or urther modalities of irradiation such as two radia tion series within a few months and high radiation volumes in cases of radiation therapy of the lym phatic system

No significant difference was found in the prog nosis between lesions of the upper and lower cervi al segments

For 36 fatal cervical radiation myelopathies a median survival time of 6 5 months is calculated ranging from ¾ to 16 5 months and for 18 fatal thoracic radiation myelopathies a median survival time of 7 5 months ranging from 2 to 15 months is found For the six fatal cases following two consecu ive irradiation cycles performed within a few months the median survival time lay at 3 (2–10) months and is particularly short

Under the hypothesis that fatal and non fatal courses or complete and incomplete transverse syn-dromes were the result of different radiation doses a possible dose effect relationship was sought Only those cases could be referred to in which the nomi nal standard dose (NSD) was either specified or could be calculated The NSD value was calculated according to the formula of Ellis

$$NSD = D \times N^{-0\ 24} \times T^{-0\ 11}$$

As the dose D was expressed in Gy the NSD value was expressed in 100 ret If the median spinal dose is calculated for those patients who died within the first 18 months and for the group of survivors (Table 1) NSD of 18 1 (median value) is yielded for the 15 fatal cervical cases and a NSD of 14 6 for the 7 survivors with incomplete transverse syndromes The difference is significant at the 10 per cent level

Almost identical NSD values of about 15 4 are calculated for the fatal and non fatal thoracic cases However if the complete and incomplete thoracic transverse syndromes are separated (Table 2) a NSD of 16 9 is found in the first group of 14 cases and a NSD of 15 3 for the second group of 6 cases This difference is statistically significant at the 5 per cent level of the Wilcoxon test For radiation myelopathies following irradiation of the lymphatic system (Table 3) a median NSD value of 15 2 is calculated for complete transverse syndromes in 4 cases and a NSD value of 13 2 for 5 incomplete transverse syndromes The difference is again sig nificant at the 5 per cent level

If incomplete transverse syndromes following up-per mantle field irradiation (Table 3) are compared with incomplete thoracic transverse syndromes

Table 2
Nominal standard dose in thoracic radiation myelopathies

Complete transverse syndrome

NSD	Authors	Case No
14 5	LOCKSMITH & POWERS (1968)	1
15	PALMER (1972)	5
15	PALMER (1972)	6
15 3	PHILLIPS & BUSCHKE (1969)	1
16 1	PALMER (1972)	1
16 7	LOCKSMITH & POWERS (1968)	3
16 7	LOCKSMITH & POWERS (1968)	6
17	ATKINS & TRETTER (1966)	12
17 5	GLANZMANN et coll (1976)	?
17 6	COY et coll (1969)	1
17 9	COY et coll (1969)	2
18 1	COY et coll (1969)	3
19 7	PHILLIPS & BUSCHKE (1969)	3
19 9	PHILLIPS & BUSCHKE (1969)	2

Median 16 9

Incomplete transverse syndrome

NSD	Authors	Case No
14 5	LOCKSMITH & POWERS (1968)	?
14 5	LOCKSMITH & POWERS (1968)	4
15	LOCKSMITH & POWERS (1968)	5
15 5	PALMER (1972)	3
16	GLANZMANN et coll (1976)	3
16 1	ATKINS & TRETTER (1966)	13

Median 15 3

Table 3
Nominal standard dose in radiation myelopathies after upper mantle field irradiation

Complete transverse syndrome

NSD	Authors	Case No
13 4	GLANZMAN et coll (1976)	5
14 9	GANSHIRT (1975)	6
15 4	GANSHIRT (1975)	9
16 ?	GANSHIRT (1975)	3

Median 15 2

Incomplete transverse syndrome

NSD	Authors	Case No
12 5	GÄNSHIRT (1975)	?
13	GLANZMANN et coll (1976)	4
13 2	GANSHIRT (1975)	4
13 4	HOLDORFF (1979)	5
13 7	GANSHIRT (1975)	7

Median 13 2

(Table 2) a median NSD value of 13 2 and 15 3 respectively is found These values differ significantly at the 5 per cent level Despite the small number of cases in both groups the statistical comparison is justifiable because all NSD values in the group following upper mantle field irradiation are smaller than the smallest value of the other group with thoracic radiation myelopathies A part of the results is compiled in Table 4

Discussion

The present results indicate that the fatal outcome of many cervical and thoracic radiation myelopathies occurs within 18 months of the appea- ance of the first neurologic symptoms i e when they develop into a complete transverse syndrome while incomplete transverse syndromes are cons[illegible] ent with a longer survival time

An attempt to distinguish upper and lower cervi- cal radiation myelopathies by morphologic and neurologic criteria does not yield any clinically use- ful differences in the course of the ultimate fatal cases The median survival time of 6 5 months for [illegible] fatal cervical myelopathies does not essentially di[illegible] fer either from the median survival time of 7 [illegible] months for 18 fatal thoracic myelopathies Nevertheless for the course and the prognosis [illegible]

Table 4

Nominal standard dose in radiation myelopathies

Cervical		Thoracic		After upper mantle field irradiation	
Fatal	18 1	Complete	16 9	Complete	15 2
Non fatal	14 6	Incomplete	15 3	Incomplete	13 2
$2\alpha<10\%$		$2\alpha<5\%$		$2\alpha<5\%$	

portance of the segmental localization of spinal croses should not be ignored

It is known that in cases of traumatic transverse sions the mortality rate depends upon the segmen l localization of the spinal lesion and increases rticularly for lesions in the upper cervical part of e medulla i e C1–C4 mainly due to acute re ratory paralysis (MESARD et coll 1978) In the ses of cervical radiation myelopathy it is difficult distinguish different courses of upper and lower rvical radiation myelopathy because of the in mogeneous distribution of the necroses over a rge segment of the spinal cord

Furthermore the present analysis proceeded on e assumption that the dose causing spinal cord ecrosis would have to differ for complete and in mplete transverse syndromes This assumption as confirmed statistically for those cases with a own NSD The median spinal necrosis dose men oned is not identical with the threshold of toler nce which lies well below the range of necrosis oses Nevertheless the spinal cord necrosis dose f incomplete radiogenic transverse syndromes omes closest to the threshold tolerance because it presents a submaximum overdosage and not a aximum one as in the case of complete transverse yndromes The scattering range of NSD is as ex ected small (Tables 2 3) For this reason incom lete transverse syndromes are best suited for de ermining the threshold of tolerance This is only ossible when the clinical course may be observed or a sufficiently long period of time so that a later rogression to a complete transverse syndrome may e excluded According to this principle a median pinal necrosis dose of NSD 15 3 and 13 2 could be etermined for the incomplete thoracic radiation yelopathies and for those following lymph node rradiation respectively

This division into complete and incomplete trans erse syndromes cannot be applied to ... es of cer vical radiation myelopathy because in many cases of the literature incomplete cervical transverse syndromes were described with an early fatal out come where presumably the complete tetraplegic transverse syndrome in the final phase was not re corded On the other hand all surviving patients presented with an incomplete transverse syndrome either unilaterally along the lines of the Brown Sequard syndrome or as a bilateral incomplete transverse syndrome Therefore it is suggested for cervical radiation myelopathies that the course criterion fatal corresponds to complete and non fatal to incomplete transverse syndromes A mean spinal necrosis dose of NSD 14 6 was determined for non fatal incomplete cervical radiation myelopathies

These criteria for neurologic differentiation have up to now not been applied in the reports on radia tion myelopathy risk According to compilations of data the critical range in which radiation myelo-pathies may occur fluctuates between NSD values of 10 and 20 (FRANKE) but most often lies above 15 (PHILLIPS & BUSCHKE 1969 GLANZMANN et coll 1976) For reasons of safety a tolerance dose 10 per cent lower than this is applied to the cervical part of the medulla (GLANZMANN et coll) In con trast ABBATUCCI et coll (1978) consider a dose of 50 Gy (25 fractionations in 35 days=NSD 15 7) to be safe if a longitudinal segment of the cervical region of the spinal cord covering no more than three to five vertebrae is irradiated For the thoracic region of the spinal cord WARA et coll (1975) calculated a 50 per cent incidence rate of approximately NSD 16 while RHEINHOLD et coll (1976) determined one of NSD 19 8 which however is probably far too high This error may be due to the fact that because of the average latency period of 18 months many cases are not recorded in morbidity investigations of radiation myelopathy because of premature death due to the primary disease (FRANKE)

Tolerance calculations do not fully appreciate the

volume factor although in principle its significance has long been known As early as 1950 BODEN found that a radiation myelopathy occurred if a longitudinal spinal cord segment of more than 20 cm was irradiated with an otherwise well tolerated dose ATKINS & TRETTER ZEMAN & SHIDNIA and ABBATUCCI et coll also stressed the significance of the volume factor It is also of importance when considering the craniospinal irradiation of children The present results emphasize that also an increased risk of radiation myelopathy is involved in high volume irradiation of the lymphatic system in cases of Hodgkin s disease and other forms of malignant lymphoreticular disease Incomplete transverse syndromes after a mantle field irradiation of this type (Table 3) and incomplete thoracic radiation myelopathies after low volume irradiation (Table 2) show a significant difference in their median spinal necrosis dose (5 per cent level) This statistical comparison is justifiable in spite of the small number of cases because all NSD values of the group after mantle field irradiation are lower than the lowest values of the other group with thoracic radiation myelopathies Thus it can be stated that the limit of tolerance of the spinal cord for mantle field irradiations is lower and a safety limit of NSD 12 may be assumed These results indicate that it would be logical to introduce the volume factor to the conventional NSD formula (GREMMEL & WENDHAUSEN 1977) especially in view of the radiation myelopathy risk

SUMMARY

The course and prognosis of radiation myelopathies are determined by 3 factors the segmental (vertical) location of the lesion the extent of the transverse syndrome (complete or incomplete) and the radiation dose The median spinal dose in cervical radiation myelopathies with fatal outcome was higher than in survivals with an incomplete transverse syndrome In thoracic radiation myelopathies a dose difference between complete and incomplete transverse syndromes could be found as well Incomplete transverse syndromes as submaximum radiation injuries are more suitable for the determination of the spinal tolerance dose than complete transverse syndromes The lowest threshold could be stated for cases following high volume irradiation of the lymphatic system

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FROM THE NATIONAL DEFENCE RESEARCH INSTITUTE DEPARTMENT 4 S 9018_ UMEÅ SWEDEN

CHROMOSOME COUNTS OF ^{90}Sr INDUCED OSTEOSARCOMAS IN MICE

IV Variation of chromosome counts when using tumours of predetermined age for transplantation

H BERGMAN

When analysing the chromosomal progression of lid tumours a critical point may be whether a rect method can be applied or whether it becomes cessary to use a sometimes less reliable in vitro chnique (SANDBERG et coll 1961 MARK 1967 SU & KELLOGG 1969) Thus in a previous report a ^{90}Sr induced osteosarcoma quite different romosome patterns were recorded when in vivo mour transplantation was performed in parallel ith in vitro cultures (Part III BERGMAN 1980) owever it must be realized that the use of an in vo method may also involve events that affect the sults As found previously in an in vivo parallel amination of two transplanted tumour series obined from two tumours with different outgrowth eriods of the second transfer generation of a ^{90}Sr duced osteosarcoma (Part II BERGMAN 1980) a onnection between the tumours used for transplan ation the age of the tumours used for chromosome nalysis and the resulting chromosome patterns ould not be eliminated The main difference con isted in a different chromosomal progression within he triploid region and a variation of the percentage f 40-chromosome cells For this reason a primary ttention of the present investigation was to examine hether a variation in chromosome count could be bserved when repeated transplantations were per ormed from different tumours of a transfer genera ion The ages of the tumours used for transplanta ion were predetermined

Material and Methods

Transplantation procedures Highly inbred 60±5 days old male mice of the CBA strain were used The primary osteosarcoma with an induction time of 403 days was induced with 14 8 kBq $^{90}Sr(NO_3)$ /g body weight By serial in vivo trans plantation (NILSSON & ULLBERG 1962) the numeri cal chromosome progression of the original fast growing transfer series designated B had been analysed previously (Part I BERGMAN & NILSSON 1980) A tumour from transfer generation B2 was used to establish a separate slow growing tumour series b (Part II) From transfer generation b10 of this series two tumour pools constituting transfer generations $b11_{(19)}$ and $B11_{(24)}$ were established The figures within parentheses indicate the age of the tumour used for transplantation to establish these two parallel generations The $b11_{(19)}$ generation is not further dealt with in the present context but will constitute the origin of an investigation of tumour transplantation to hyperimmunized mice In the pres ent investigation repeated transplantation was car ried out from transfer generation $b11_{(24)}$ (Fig 1) on predetermined days i e tumour material in this case was harvested for transplantation on days 11 18 29 44 and 63 after the day when the $b11_{(24)}$ generation was established Thus from the five tumours used

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for transplantation five transfer generations b12 were obtained From each of these generations transplantation was performed by using a tumour with an outgrowth period that was identical to the age of the tumour used to establish generation b12 This means for example that from transfer generation $b12_{(11)}$ a tumour with an outgrowth period of 11 days was used to establish the succeeding transfer generation designated $b13_{(11)}$ In order to permit comparison with the original fast growing transfer ... B transfer generations B11 to B13 are presented also in the present investigation

Chromosome analysis A detailed description of the method used for preparation of chromosome ... has been presented previously (Part I) ... ther five tumours per transfer generation were examined but as it is still difficult to obtain successful chromosome preparations from these tumours only 25 well spread metaphases per tumour were analysed Due to the rather few chromosome counts ... tumour only the percentile chromosome distribution per transfer generation is presented

Histologic examination The definition of tumour classification and the method used have been described in Part I as well as the histologic characterization of tumours from the original B series However in the present investigation a tumour from each transfer generation of the b series was used for histologic examination Concerning the

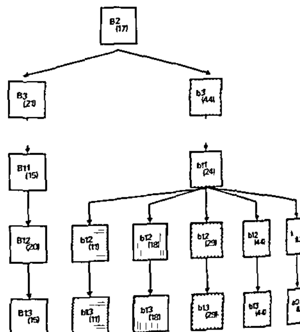


Fig 1 Diagram of the transplantations Figures within parentheses indicate age of the tumour used for transplantation to establish a particular generation

tumours of the b12 generations slight indications were found of a more marked bone formation in la... established transfer generation Finally no dis... variation was observed at histology betwe... tumours of different b13 generations but a v... from tumour to tumour from moderate bone forma... tion to anaplasia

Table

The percentile chromosome distribution of the transfer generations examined The figures within parentheses indicate the age of the tumour used to establish a certain transfer generation

Transfer generation	No of tumours examined	No of cells counted	Chromosome number 39	40	41	42	43	44	45	46	47	48	49	50	51	5…
B11	5	125	0 8	86 4	1 6											
$b11_{(24)}$	5	125		16 0											2 4	3 2
$B12_{(20)}$	5	125		72 8												
$b12_{(11)}$	5	125	3 2	28 0									0 8	0 8	0 8	7 2
$b12_{(18)}$	5	1 5	0 8	17 6									0 8		1 6	1 0
$b12_{(29)}$	5	125	0 8	16 8	0 8				0 8	0 8	0 8	0 8			1 6	11 6
$b12_{(44)}$	5	125		10 4				1 6	0 8			0 8		1 6	4	10
$b12_{(43)}$	5	1?5		14 4							0 8	0 8		1 6	1 6	10 4
$B13_{(15)}$	5	1 5	0 8	67 2												
$b13_{(11)}$	5	125	4 0	62 4										1 6	3 2	3
$b13_{(18)}$	5	1 5	0 8	49 6											0 8	0 8
$b13_{(29)}$	5	125	2 4	40 0						1 6	0 8				0 8	8 8
$b13_{(44)}$	5	125	0 8	20 8		0 8						1 6		3 2	7 4	4 4
$b13_{(42)}$	5	125	0 8	23 2	0 8		0 8								1 6	4 6

Mean growth period

11 12 13

a

Mean size

150 100 0.50

11 12 13

b

Mean growth per day

0.100 0.080 0.060 0.040 0.020

11 12 13

Transfer generation

c

Fig 2 Mean growth period (days) mean size (cm) and mean growth per day (cm³) of the tumours per generation Figures within parentheses indicate age of the tumour used to establish a particular transfer generation ○B11 +B12 +B13 ⊗b11 ● b12 +b13 □ b12 +b13 ■ b1 +b13 △ b12 +b13 ▲ b12 +b13

Results

Recording of chromosome abnormalities As in previous investigations of ⁹⁰Sr induced transplanted tumours the recording of abnormalities was limited to numerical chromosome deviations and the occurrence of metacentric configurations (Parts I II III) Thus frequently appearing temporary structural abnormalities were not recorded

A diagram of the transplantations is presented in Fig 1 and the mean outgrowth mean size and mean growth per day of the tumours per transfer generation in Fig 2 The percentage of metacentrics appears in Fig 3 while Figs 4 to 6 show cells with different chromosome numbers The percentile chromosome distribution per transfer generation is presented in the Table

Table (cont)

54	55	56	57	58	59	60	61	6?	63	64	84	10?	103	104	105	106	107	108
0 8																		
3?0			0 8	0 8		1 6	4 8	2 4										
					2 4	12 0	10 4	_4										
17 6	0 8																	
3??	5 6																	
16 0	3?	4 8	0 8															
40 0	7?	1 6	0 8		0 8											0 8	0 8	
1 8	13 6	6 4	10 4	3 _	1 6													
6 4		0 8	0 8	0 8	1 6	1 0	1_8	2 4	0 8									
18 4	3?								0 8	0 8	0 8	1 6	4	1 6	0 8	1 6	0 8	3 2
14 4	8 0	0 8	0 8											1 6	0 8	0 8		
33 6	16 0	4 8	0 8	1 6														
1?8	34 4	11?	3 2	0 8														

Chromosome distribution The tumours of the original B series (transfer generations B3–B10) (Part I) were primarily characterized by a varying percentage of 40 chromosome cells (22 4–73 6%) in combination with cells within the triploid region Within this region a predominating number of 58 to 63 chromosome cells was found

The transfer generations b3–b10 obtained by separate serial transplantations from a tumour of transfer generation B2 (Part II) differed from the tumours of the B series within the triploid region cells with almost continuously lower chromosome numbers were recorded i e a changed predominance from 57 chromosome cells in generation b3 to 53 to 54-chromosome cells in b10 Furthermore the b generations displayed an often lower percentage of 40 chromosome cells (4 2–59%) than the B generations

Transfer generation $b11_{(21)}$ constituting a tumour pool for the five consecutive transplantations displayed a predominant number of cells within the 51 to 54 region and with peaks at 53 (46 4%) and 54 (32%) Besides this distribution 16 per cent 40 chromosome cells were found In contrast to these results the original B series showed a predominance of 40 chromosome cells (86 4%) and a small number of cells within the 60 to 62 region

A certain difference found between the five b12 generations was the varying percentage of 40-chromosome cells The percentage of 40-chromosome cells gradually declined from transfer generation $b12_{(11)}$ (28 0%) to generation $b12_{(44)}$ (10 4% Table) The $b12_{(63)}$ generation diverged slightly from this pattern as it was characterized by 14 4 per cent 40-chromosome cells It was also observed that while the $b12_{(11)}$ generation displayed a predominating number of cells within the 52 to 54 region the $b12_{(63)}$ generation in particular varied somewhat more i e from 52 to 58 The B generation was still characterized by a high frequency of 40-chromosome cells (72 8%) but also by an increased number of cells within the 60 to 62 region

The chromosome counts of the b13 generations revealed an increased number of 40-chromosome cells Similarly to the preceding generations (b12) most 40-chromosome cells were observed in the early transplanted generations and except for generation $b13_{(63)}$ a continuously lower percentage was observed (from 62 4% for $b13_{(11)}$ to 20 8% for $b13_{(44)}$) It was also found that the early transplanted generations still showed a narrower range of variation

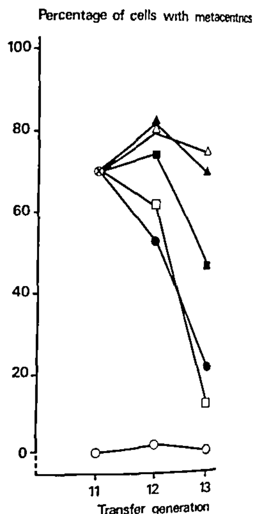


Fig 3 Percentage of cells with one or more metacentrics Sy bols as in Fig 2

within the 50 to 60 region In the B series finally continued predominance of 40-chromosome cell (67 2%) was found and within the triploid region range of 56 to 63

Metacentric chromosomes The tumours of th B series displayed only 0 8 per cent cells with one o more metacentrics while within the b-series a var ing percentage ranging from 11 5% (generatio $b13_{(18)}$ to 80 9% (generation $B12_{(63)}$) was found (Fi 3) Concerning transfer generations b12 the percen tage increased continuously from $b12_{(11)}$ to b1 i e from 51 9 to 80 9 per cent Even if a genera smaller number of cells with metacentrics (11 74 2%) was recorded from the b13 generations th late established generations like the b1 genera tions showed a higher percentage However must be realized that these results are biassed by th number of 40-chromosome cells found these ce very rarely displayed metacentrics

Mean outgrowth period The tumours of th

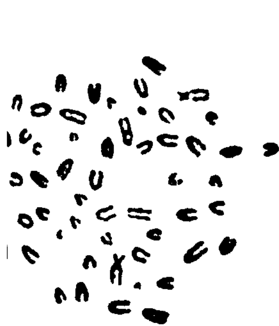


Fig 4

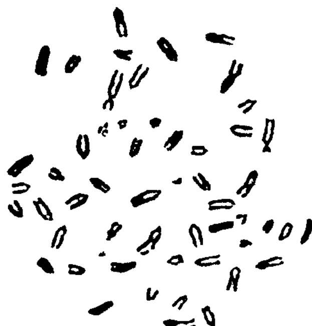


Fig 5

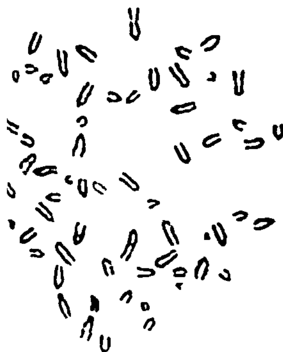


Fig 6

Fig 4 A 53 chromosome cell from a tumour of transfer generation b1? ,

Fig 5 A 54-chromosome cell from a tumour of transfer generation b13 ₄₄

Fig 6 A 62-chromosome cell from a tumour of transfer generation B11

ternal B series were characterized by the shortest mean outgrowth period per generation (14 6–16 0 days Fig 2) Regarding the b-series a generally prolonged mean outgrowth period was observed which means that the early established generations also contained the fastest growing tumours

Conclusions

As in previous investigations of primary tumours fast growing tumours showed normal chromosome numbers while slower growing tumours also displayed abnormal chromosome numbers (KATO 1968 MARK 1969 MITELMAN 1971 1972) In a previous

report on transplanted ^{90}Sr induced tumours heterogeneous chromosomal progression was obtained when two tumours of a transfer generation were used for separate serial transplantation (Part II) An attempt has now been made to examine whether the age of tumours used for transplantation may be of importance for the succeeding chromosomal evo lution and for the outgrowth period of the trans plantates For this reason repeated transplantations were performed from different tumours of transfer generation b11 When comparing the B and b-series it was found that the main differences in chromosome distribution observed in earlier transfer generations were maintained (Part II) This means that the B generations in general were characterized by more 40-chromosome cells and within the 50 to 62 region a predominating number of triploid or hypertriploid cells in contrast to the b-series which had a majority of 52 to 56-chromosome cells Thus in spite of a forced transplantation from early outgrown tumours of transfer generation b11 (to obtain transfer generations $b12_{(1)}$ and $b12_{(?)}$ and then also to establish the consecutive generations $b13_{(1)}$ and $b13_{(16)}$) no regression could be observed to the characteristic and stable 60 to 62 region found in the fast growing tumours of the B series However the early established b12 and b13 generations displayed a higher frequency of 40-chromosome cells and an almost continuosly lower percentage with increasing age of the tumour used for transplantation (Table) This result may thus indicate a connection between the age of the tumours used for transplantation and the chromosomal progression but it must be realized that a quite normal accidental variation may cause this distribution This may also explain the variation found within the 50 to 60 region between early and late established generations Even if the mean out growth per day and mean outgrowth period per gen eration (Fig 2) should be evaluated with a certain caution a true relationship between the age of the tumours used for transplantation and the outgrowth period of the transplantates cannot be eliminated

SUMMARY

Transplanted tumours from a ^{90}Sr induced primary tumour were used as an experimental model to eliminate any connection between the chromosome distribution and outgrowth period of radiation induced transplanted tumours For this reason repeated transplantations were performed from different tumours of predetermined age from a transfer generation to receive separate but p[illegible] transfer generations From each one of these generations a tumour of the same age as used to establish a particular generation was used for continued transplantation It was then observed that the percentage of 40-chromosome cells in general decreased with the age of the tumour used for transplantation Furthermore early established transfer generations were often characterized by more slow growing tumours However it should be realized that even if a true relationship between chromosome pattern and outgrowth period cannot be eliminated the differences found may be due to accidental variations.

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FROM THE DEPARTMENT OF RADIATION BIOPHYSICS FACULTY OF MEDICINE UNIVERSITY OF TOKYO TOKYO JAPAN

RADIATION SENSITIVITY OF DNA MOLECULES IN SITU IN NORMAL AND NEOPLASTIC TISSUES OF MICE

TETSUYA ONO KIYOHIKO SAKAMOTO and SHIGEFUMI OKADA

Sensitivity to radiation in various tissues in man and in animals is known to vary a great deal depending upon the type of tissues (CLEMENDSON & NELSON 1960) In a previous report on air breathing live mice one sensitive tissue the thymus and one resistant tissue the liver were found to show similar in situ sensitivities in their DNA molecules (ONO & OKADA 1974) This raised the question whether all DNA molecules in all tissues of mice may have the same sensitivity regardless of the tissue sensitivity The purpose of the present investigation was to extend the previous work into two other normal tissues (spleen cerebellum) and one malignant tissue (a squamous cell carcinoma) and to assess the role of oxygen by comparing the DNA sensitivities in various tissues from air breathing live mice and nitrogen asphyxiated dead mice The nitrogen asphyxiated death was used to create a hypoxic state in tissues of animals as previously demonstrated by CATER (CATER & PHILLIPS 1954 CATER 1960) and used in animal experiments by DESCHNER & GRAY (1959) and HEWITT & WILSON (1959 1961)

Materials and Methods

WHT/Ht mice aged 9 to 14 weeks were used throughout the experiments Each mouse was confined in a plastic box (4 0 cm×4 0 cm×4 5 cm) and irradiated at 33 Gy/min (measured by Fricke s ferrous sulfate dosimeter) with gamma rays from a 148 TBq of ^{137}Cs source (the Atomic Energy Research Center University of Tokyo) For hypoxic irradiation mice were killed by nitrogen asphyxiation kept at room temperature (5 20 C varied with the season) from 30 to 80 min (CATER & PHILLIPS CATER) to allow tissues to become hypoxic and irradiated at room temperature Under these experimental conditions the sedimentation profiles of DNA were the same independent of the time between killing and irradiation and of room temperature

Within 3 min after whole body irradiation tissues were removed and soaked in ice cold Fischer s medium with 10 per cent horse serum Tissues (spleen thymus and cerebellum) were cut into pieces with a pair of scissors and sieved through stainless mesh of pore size of 125×125 microns A cerebellar cell suspension was centrifuged once or twice at 50 G for 1 min to remove large capillary debris

A squamous cell carcinoma transplanted subcutaneously in armpits of mice (HEWITT et coll 1967 HEWITT & SAKAMOTO 1971) was used Most tumours were from 0 1 to 0 3 g although occasionally tumours as large as 1 2 g were used and necrotic parts were removed with a pair of forceps as much as possible The remainder of the tumour was then dispersed to prepare a cell suspension as described Finally tumour cells were spun down at 400 G for 2 min and resuspended Liver cell suspensions were prepared by the method of BRANSTER & MORTON (1957)

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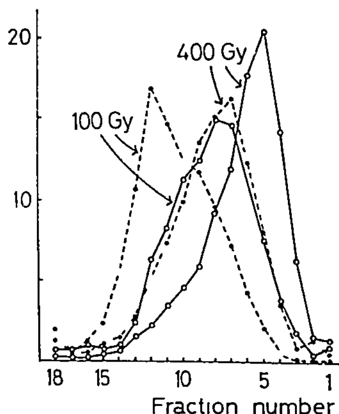


Fig 1 Alkaline sucrose gradient sedimentation profiles of spleen DNA after 100 Gy and 400 Gy of whole body irradiation in situ under oxygenated (living) or hypoxic (dead) conditions Sedimentation direction is from right to left Solid lines with open circles indicate DNA irradiated under oxygenated conditions and the dotted lines with closed circles under hypoxic conditions Each profile represents one spleen from one animal The profiles here are four typical samples The profile (not shown) of splenic DNA from a non irradiated animal overlapped with that of the profile with 100 Gy under hypoxic conditions shown in this figure

A cell suspension was adjusted to a cell concentration of 4 to 8×10^6 cells/ml A half ml of the suspension was gently layered over a sucrose gradient solution in a nitrocellulose centrifuge tube The sucrose gradient was made by placing 1 5 ml of a 25 per cent sucrose solution on the botton of a nitrocellulose centrifuge tube as a cushion followed by 34 ml of a 20 to 5 per cent sucrose gradient (0 9 M NaCl 0 1 N NaOH and 0 01 M Na_2EDTA) and finally 0 5 ml of a lysing solution (0 5 N NaOH and 0 1 M Na_2EDTA) as a top layer (ONO & OKADA 1974) For liver cells 0 3 ml of a cell suspension was applied over 0 7 ml of the lysing solution on the sucrose gradient

After layering a cell suspension on the sucrose gradient the gradient was kept at 29 °C for 3 hours to complete lysis Then they were centrifuged at 25000 rpm for 165 min in a SW 27 rotor in a Beckman L3 50 Ultracentrifuge For liver cells the centrifugation time was 270 min The DNA sedimentation profile was analyzed by a fluorometric method (ONO & OKADA 1973) and the average molecular weight of DNA was calculated as described previously (ONO & OKADA 1974)

Results and Discussion

The alkaline sucrose gradient centrifugation patterns of splenic DNA after 100 Gy and 400 Gy of irradiation under oxic or hypoxic conditions appear in Fig 1 The number average molecular weights of the data in Fig 1 were 6 3×10^7 daltons under oxic and 11 1×10^7 daltons under hypoxic conditions after 100 Gy and 3 1×10^7 and 6 3×10^7 daltons under oxic and hypoxic conditions after 400 Gy of gamma irradiation The sedimentation profile of the non irradiated control (not shown in Fig 1) was very similar to that of 100 Gy under hypoxic conditions in Fig 1 and its average molecular weight was 1 3×10^8 daltons The reciprocals of the number average molecular weights which were proportional to the number of single strand scissions were plotted against the radiation dose (Fig 2a) The regression analysis of normal and hypoxic experiments reveals that the efficiency of DNA breakage is 60 single strand breaks/10^{12} daltons DNA/Gy (or 172 eV/s s b) under normal conditions and 19 single strand breaks/10^{12} daltons DNA/Gy (or 545 eV/s s b) under hypoxia Fig 2b to d shows the results with thymus cerebellum and liver The dotted lines in Fig 2b and 2d under oxic conditions are based on the previously reported data (ONO & OKADA 1974) with some additional points All other experiments are new data where each point represents one tissue from one mouse

In the liver (ONO & OKADA 1974) no data at doses over 400 Gy under normal conditions were available because most mice exposed to the higher doses died during the irradiation The breakage efficiency of hepatic DNA under hypoxic conditions (41 s s b/10^{12} daltons/Gy or 252 eV/s s b) showed a slight reduction from those (62 s s b /10^{12} daltons/Gy or 167 eV/s s b) under normal conditions in contrast to the results of spleen thymus and cerebellum It is stated that the time required to rejoin 50 per cent of single strand breaks range from 40 min to 2 hours in all the normal tissues analyzed thymus and liver (ONO & OKADA 1974) and cerbellum and spleen (ONO & OKADA 1978) Thus the extent of DNA

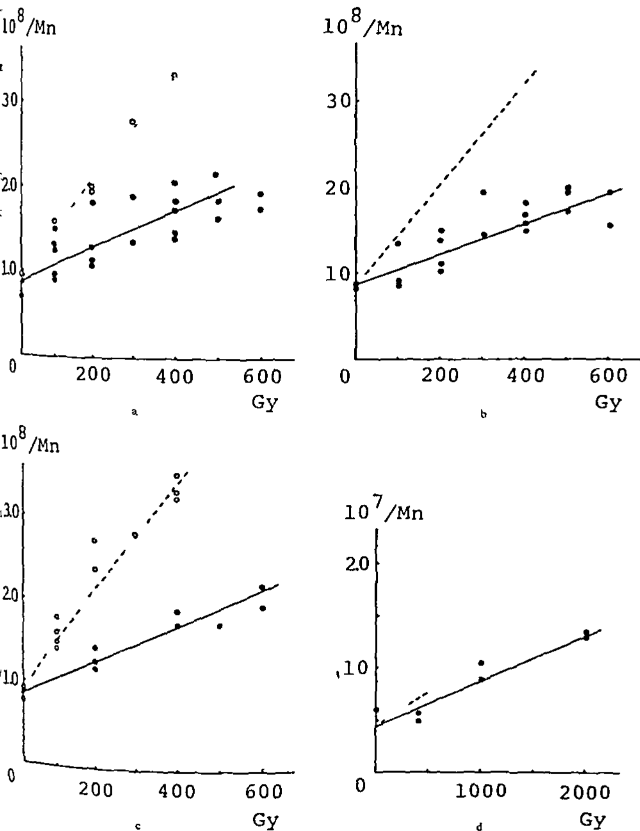


Fig. Dose response curve for single strand breaks in a) spleen b) thymus c) cerebellum and d) liver DNA in situ. The reciprocal of the number average molecular weight which is proportional to the number of single strand breaks is plotted against dose. Each point represents one animal. The lines represent the regression curve. The dotted line with open circles is that of the oxic conditions and closed circles that of the hypoxic conditions. Dotted lines in (b) and (d) are the results of regression analysis with some additional data to the previously published curves (ONO & OKADA 1974)

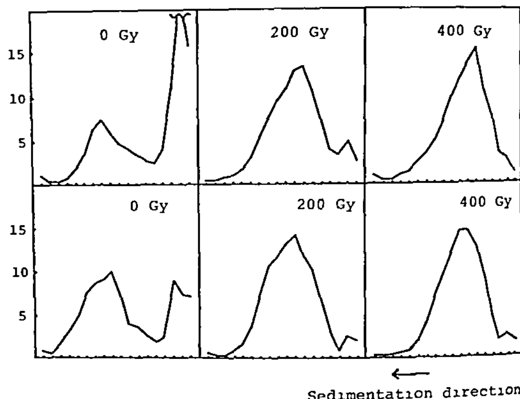
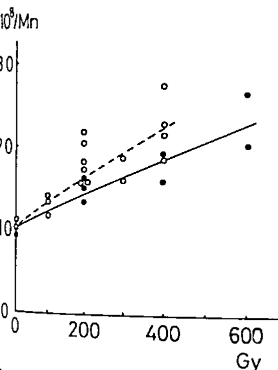


Fig 3 Sedimentation profiles of irradiated or non irradiated tumour DNA under oxic (upper curves) or hypoxic (lower curves) conditions Tumour volume was 0 1 to 0 3 g Left top panel is the sedimentation pattern of tumour DNA without a centrifugation procedure for purification of the cell population before lysis of cells Top three fractions were neglected for the calculation of average molecular weight

scissions rejoined during and after irradiation till death would amount at most to 8 per cent In other words the observed break efficiencies would be more than 92 per cent of the initial breaks induced by the irradiation

It is added that the DNA size in liver of non irradiated mice was found to be significantly lower than those observed in other tissues with exception of spermatozoa (ONO & OKADA 1977) Previously (ONO & OKADA 1974 1978 ONO et coll 1977) several attempts have been made to find whether autolysis of DNA during cell preparation and other steps would cause a reduction of DNA size For example a DNAase inhibitor was added during the cell preparation (ONO & OKADA 1978) Or the time interval between cell preparation and cell lysis was altered (ONO & OKADA 1974 ONO et coll) None of these affected the DNA size Moreover, the DNA size of old mice was found to be smaller than that of young adult mice (ONO et coll ONO & OKADA 1978) Thus the small DNA size might be real It is also pointed out that the estimation of DNA breakage efficiency under oxic conditions is similar to those of other tissues and that the estimation of the breakage efficiency obtained from the slope of the dose response curve would not be affected by the small DNA size of non irradiated liver

In a squamous cell carcinoma the sucrose gradient pattern of DNA was made up of one major peak in the center of the gradient and another peak near the top of the gradient The top peak represent DNA of molecular weight of less than 10^{8} dalton found in the top three fractions of the gradients The DNA content varied greatly from one sample to another A typical example is shown in the top panel of the left column of Fig 3 (oxic 0 Gy) Since the DNA may have come from autolysis of cells in necrotic region of tumours (ONO & OKADA 19 WILLIAMS & LITTLE 1975) a cell suspension was centrifuged at 400 G for 2 min several times before applying it to a sucrose gradient The procedure removed most of the top peaks of low molecular weight DNA (cf the rest of panels in Fig 3) When calculating the average molecular weight the top three fractions were excluded Fig 4 shows that the dose response curve of tumour DNA from live ... was slightly higher than that of nitrogen asphyxia mice It is added that the size of tumours (0 1 g to 1



ig 4 Dose response curve of tumour DNA in situ under oxyge ated (O) and hypoxic (●) conditions Each point represents one nimal The lines are the results of regression analysis The andard deviations for oxic and hypoxic conditions appear in the able

) seemed not to influence the break efficiency of imours

Since the rejoining time of the single strand breaks f the tumour is unavailable the extent to which the reak efficiencies estimated for tumours would be ffected by a possible rejoining during and after ir adiation is unknown However it may be specu ated that the observed break efficiencies of tumours as 70 per cent or more of the initial breaks induced y irradiation from the following considerations (1) f the rejoining rate of the tumour is as slow as those f 4 normal tissues (ONO & OKADA 1974 1976 1978) he observed efficiencies would represent more than ? per cent of the initial breaks (2) If the rejoining is s fast as that (the half time of 10 min at 100 Gy) of estis (the fastest among 5 tissues in this strain of uice ONO & OKADA 1976) the observed breaks ould be 72 per cent of the initial breaks (3) The arcinoma is known to contain an hypoxic compo ent to a significant extent (HEWITT et coll IEWITT & SAKAMOTO) Such a hypoxicity might low the rejoining rate further (MODIG et coll 1974) f so more than 72 per cent of the initial breaks night be accounted for as the observ d breaks

One of the target molecules re nsible for the reproductive death of mammalian cells is probably DNA (OKADA 1971a) Several experiments have been reported in which a correlation between the DNA break efficiencies and cellular radiation sen sitivity is demonstrated Some of them are oxygen effect (MODIG et coll LENNARTZ et coll 1973 CHAPMAN et coll 1974) bromodeoxyuridine sen sitization of radiation (SAWADA & OKADA 1971) and protection by cysteamine and other compounds (SAWADA & OKADA 1970 ROOTS & OKADA 1972 ROOTS & SMITH 1974) Since at least two modes of cell death reproductive and interphase death (OKA DA 1971b) are known to occur in somatic cells of various tissues this brings about the question how the sensitivity of DNA molecules in various tissues would relate to the modes of cell death The present results are summarized in the Table They show that DNA sensitivity in normal tissues of live mice are all in the same order of magnitude and that there is no relationship of DNA sensitivity to cellular prolifera tive capacity or to tissue sensitivity or the modes of cell death

The DNA sensitivity of tumours in mice were found to be significantly lower than those of 4 nor mal tissues even after a maximum correction of a possible fast rejoining

In nitrogen asphyxiated mice the radiation sen sitivity of normal tissues with the exception of liver and of the carcinoma was found to have decreased to the same level of 17 to 22 Since nitrogen asphyxiation makes tissues hypoxic (DESCHNER & GRAY CATER & PHILLIPS CATER) and since the squamous cell carcinoma in situ is found to contain a significant portion of hypoxic cells (THOMLINSON & GRAY 1955 HEWITT et coll HEWITT & SAKAMOTO) the relatively low DNA sensitivity of the squamous cell carcinoma in live mice could be attributed to its low hypoxicity In other words hypoxicity of the tumour seemed to be demonstrable at molecular level

In the squamous cell carcinoma the oxygenated portion was estimated by the TD50 method to be around 82 per cent and the hypoxic portion about 18 per cent (HEWITT et coll HEWITT & SAKA MOTO) If it is assumed that the break efficiency of DNA in oxygenated tumour cells is 60 and 22 in hypoxic cells (Table) the observed break efficiency of 29 in carcinomas of air breathing mice would give an oxygenated component of 18 per cent and a hypoxic component of 82 per cent The ratio of the oxygenated versus hypoxic components by the

Table

Comparison of DNA radiation sensitivity in various tissues of the mouse under oxic and hypoxic conditions. DNA radiation sensitivity in eV/s.s.b. units can be obtained by dividing 10 360 with the number of s.s.b./10¹² daltons of DNA/Gy

Organ	Number of single strand breaks/10¹² daltons of DNA/Gy (±SD)		OER (±SD)
	Oxic	Hypoxic	
Spleen	60± 7	19±1	3.2±0.4
Thymus	59± 5	17±3	3.6±0.7
Cerebellum	58± 8	21±3	2.8±0.5
Liver	62±16	41±4	1.5±0.4
Tumour	29± 4	22±4	1.3±0.3

TD50 method seems to be quite different from the values estimated by the present DNA break efficiency assay. One possible explanation for the difference is that the TD50 method is based upon the clonogenicity of tumour cells, while the DNA sedimentation method would include clonogenic and non-clonogenic cells for their measurement. The difference in the estimation of oxygenated and hypoxic components by two methods may be evaluated by assuming a high cloning efficiency of the oxygenated component versus a low cloning efficiency of the hypoxic component. For example, if a relative cloning efficiency of 1 is assigned to the oxygenated component, the relative cloning efficiency of the hypoxic component would be 0.048 (0.18×0.18/0.82×0.82) or 4.8 per cent of the oxic component.

The oxygen enhancement ratios of normal tissues were estimated from comparison of DNA sensitivity of air breathing mice and nitrogen asphyxiated dead mice. The oxygen enhancement ratios of spleen, thymus and cerebellum were found to be about three (Table). They were comparable to the ratios estimated from DNA single strand scissions in cultured mammalian cells (CHAPMAN et coll., MODIG et coll.), rat thymocytes in vitro (LENNARTZ et coll.) and mouse testicular cells in vitro (ONO & OKADA 1976). The oxygen enhancement ratios of tissues in situ have been previously estimated only in a limited number of tissues in which either clonogenicity or cell loss could be measured. For example, intestinal crypt cells gave a ratio of 2.6 (HORNSEY 1970), the spleen 2.1 (HORNSEY 1967), the testis 1.8 to 2.5 (HORNSEY et coll. 1971), etc. The present method utilizing alkaline sucrose gradient centrifugation offers another means to estimate the oxygen enhancement ratios of various tissues in situ. Th[e] method can be applied to any tissue in which th[e] present sucrose gradient method is applicable.

A low oxygen enhancement ratio of liver is som[e]what different from those of tumour and testis (ON[O] & OKADA 1976, 1977). The low ratio in liver i[s] attributed to a high DNA scission efficiency of th[e] hypoxic condition, while those of tumour and testi[s] are attributed to low efficiencies under the oxic con[di]ditions. It is possible that the liver, being full of re[d] blood cells, would be one organ difficult to make hypoxic.

SUMMARY

The sensitivity to radiation of DNA molecules in si[tu] in various tissues was estimated by alkaline sucrose gradi[ent] centrifugation. The sensitivity of DNA in liver, thym[us], spleen and cerebellum was found to be of the same order of magnitude. The oxygen enhancement ratios of DNA in spleen, thymus and cerebellum were all approximately three. Sensitivity of DNA molecules in the tumour was about half of that in normal tissues, probably because of its hypoxicity.

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INDUCTION OF NEOPLASIA BY ^{140}Ba IN MICE

A NILSSON P BIERKE and A BROOMÉ KARLSSON

The uptake and retention of ^{133}Ba and ^{140}Ba–^{140}La 1 mouse tissues was investigated by DENCKER et oll (1976) It was found that the initial distribution f radiobarium was similar to that of ^{90}Sr with a igh but less specific uptake in the skeleton This vas evidenced by the fact that barium still could e autoradiographically discernible in numerous soft issues after 2 days or more whereas strontium lready after 4 hours is observable only in the hard issues (NILSSON & ULLBERG 1962) In pigmented issues the Ba activity seemed to be almost as high nd long lasting as in the skeleton Except for these lifferences radiobarium and radiostrontium have an lmost identical distribution particularly within the keleton

Unlike ^{90}Sr ^{140}Ba has a very short physical half ife (12 8 days versus 28 years) therefore offering a ood opportunity for evaluating the cancerogenicity after a relatively short initial irradiation as compared with nuclides giving a life long irradiation On the as sumption that a minimum time and thus a minimum accumulated dose is necessary for induction of histologic changes successively leading to tumours it seems probable that the dose delivered after the time when autonomy has been acquired is of little importance or may even have a depressing effect on tumour development The role of this wasted irra diation in tumour induction after contamination with long lived nuclides is debated and not fully understood The purpose of the present communica tion is to give more information about ne tumour incidence in relation to dose and sex after treatment with ^{140}Ba and a better knowledge about the role of wasted irradiation for the frequency and multi centric genesis of osteosarcomas

Material and Methods

Male and female C_{57}Bl mice (bred at the Swedish Institute of National Defence Sundbyberg) were injected intraperitoneally with ^{140}Ba nitrate when 75±5 days old and weighing 25±2 and 23±2 g respectively Three different doses—55 5 (1 5) 37 0 (1 0) and 18 5 (0 5) kBq (μCi)/g body weight—were used plus untreated controls as recorded in Table 1 The mice were housed in conventional animal rooms 10 in each cage with free access to a pelleted diet (Standard Feed for Rats and Mice Astra Ewos Sodertalje Sweden) and water The room temperature was 22 to 24 C relative humidity 45 to 55 per cent and the light/dark cycle 12/12 h Visual health control was performed twice daily and mori bund animals examined roentgenologically in dorso ventral position The films were used as a guide for locating bone tumours at the post mortem The car cases were weighed as well as the thymus spleen adrenal glands liver and the testes These organs as well as neoplastic tissues and both femora tibiae humeri sternum pelvic bones and the spine were fixed in Stieve s fluid The bones were decalcified in formic acid 20% Ordinary histologic techniques were used and all sections were stained according to

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Table 1

C_5 Bl mice injected intraperitoneally with [14] Ba nitrate

Inj amount of [140]Ba/g b w		Males		Females	
kBq	(μCi)	No	Group	No	Group
55.5	(1 5)	50	A	50	B
37 0	(1 0)	50	C	50	D
18.5	(0 5)	50	E	50	F
0 0	(0 0)	70	J	70	K

the van Gieson method and with haematoxylin and eosin All tumo rs v ere classified in agreement with the nomencla r recommended by the Committee on Patholo h European Late Effects Project Group (EULE)

Results

The num r sarcomas and mean survival times are in Table 2 In the highest and intermed ups the tumour incidence was approxim ma 3 times higher in the female than in groups of mice whereas in the lowest ups the tumour rate was the same As rega the relationship between dose and tumour ate dose dependency is evident particularly if the whole material is taken into consideration giving 32 21 and 10 osteosarcomas in the 55 5 37 and 18 5 kBq groups respectively On the other hand the dose does not seem to much influence the induction time As regards the tumour multiplicity only the highest dose level indicates a tendency to the occurrence of multiple tumours (1 18) among the tumour bearing mice

Of a total of 63 osteosarcomas in the whole material 48 (76 2%) were classified as osteoblastic osteosarcomas (Table 3) The predominance of this tumour type is a general feature in all dose groups Besides the osteosarcomas found it should be pointed out that 3 cementifying fibromas were found in the alveolar bone of the male mice given 37 kBq Also in the female control material two bone tumours one osteosarcoma and one osteoma appeared The femur and tibia as well as the lumbar vertebrae were the bones most frequently involved in tumour induction (Table 4) In the 55 5 and 37 kBq female groups 59 and 44 per cent of the tumours respectively were located in the long bones

Lymphoreticular (LR) tumours of the material had a slightly higher incidence female (51 4%) than male mice (41 4% T In the two highest [140]Ba groups the frequ LR tumours was less or equal to that of the groups whereas the incidence in the lowe group was approximately the same or slightl than in the controls Considering the site a of LR tumours (Table 3) many of these invol mesenteric lymph nodes and histological sented the characteristics of reticulum cell s type B (DUNN & DERINGER 1968)

In other cases the liver was the main or volved together with the uterus and vagi occasionally other organs as well These t were obviously identical with reticulum cell s type A (DUNN & DERINGER) When th was the only organ affected the diagnosis cell sarcoma was preferred Malignant lym were sometimes found which only invol thymus

Discussion

In a previous investigation of [140]Ba expos (DENCKER et coll) the following approxi radiation doses were found sternum 0 061 0 166 and lumbar vertebrae 0 101 Gy per kBq) of [140]Ba injected intraperitoneally In these figures are inserted in order to calcul approximate irradiation doses which wer mulated in femur lumbar vertebrae and ster the 3 different dose groups of [140]Ba used present investigation

As stated by DENCKER et coll [14]Ba pr bone tumours at significantly lower dose leve observed for [90]Sr in the CBA mouse In the dose groups (55 5 kBq) i e the groups whic exposed to a dose of 6 Gy to the femur 3 6 lumbar vertebrae and 2 2 to the sternum (Ta 37 per cent of the females and 18 per cent males developed osteosarcomas In this dose there was a total of 32 bone tumours if mal females were taken together of which 13 (were located in the femora + tibiae i e bones have accumulated a dose of approximately Nine (28 1%) were found in the spine whic calculated to have received about 3 6 Gy w no neoplasm was detected in the sternum

In the intermediate dose groups (4 2 4 and

Table 2

Incidence of malignant bone tumours and lymphoreticular tumours in relation to dose of Ba

Group of mice	Inj amount of ¹⁴⁰Ba/g b w kBq	(μCi)	No of mice	Survival days (mean ± SE)	No of mice with osteosarcoma	Survival days (mean + SE)	Total No of osteosarcomas	Lymphoreticular tumours No	Per cent	Survival days (mean ± SE)
(M)	55 5	(1 5)	50	603±23 9	9	772±36 1	10	15	30 0	585±21 8
(F)	55 5	(1 5)	48	590±19 4	18	614±23 2	22	15	31 3	561±31 6
(M)	37	(1 0)	49	712±21 8	5	686±100 4	5	21	42 9	707±29 5
(F)	37	(1 0)	48 *	604±26 3	16 *	678±41 7	16	19	39 6	572±55 9
(M)	18 5	(0 5)	49	690±22 6	5	766±51 9	5	24	49 0	675±18 6
(F)	18.5	(0 5)	49	661±24 3	5	864±54 9	5	26	53 1	640±31 9
(M)	0 0	(0 0)	70	653±21 5	–	–	–	29	41 4	641±24 3
(F)	0 0	(0 0)	70	681±19 3	1	462	1	36	51 4	648±18 0

One mouse lost during the experiment

Two mice lost during the experiment

* Including one angiosarcoma of the bone marrow

Table 3

Classification and incidence of Ba induced tumours

Type of tumour	Injected amount of ¹⁴⁰Ba kBq (μCi)/g b w: 55 5 (1 5) Male n=50	55 5 (1 5) Female n=48	37 (1 0) Male n=49	37 (1 0) Female n=48	18 5 (0 5) Male n=49	18 5 (0 5) Female n=49	0 0 Male n=70	0 0 Female n=70
Osteosarcomas								
Osteoblastic	6	19	5	11	2	4	–	1
Fibroblastic	1	3	–	1	1	–	–	–
Chondroblastic	–	–	–	–	1	1	–	–
Teleangiectatic	3	–	–	–	–	–	–	–
Mixed	–	–	–	3	1	–	–	–
Angiosarcoma of bone marrow	–	–	–	1	–	–	–	–
Cementifying fibroma	–	–	3	–	–	–	–	–
Osteoma	–	–	–	–	–	–	–	1*
Lymphoreticular tumours								
Malignant lymphoma	3	7	5	5	10	3	6	8
Reticulum cell sarcoma								
Type A	3	3	4	3	4	8	6	10
Type B	8	5	9	9	7	12	12	14
Plasmacytoma	–	–	–	–	–	–	1	1
Unclassified	1	–	3	2	3	3	4	3

* Osteoma in nasal bone survival time 1030 days

to the femur lumbar vertebrae and sternum respectively) the frequency of osteosarcomas was 33 3 per cent for females and 10 2 per cent for males Totally 21 tumours were detected of which 7 (33 3%) were sited in femur + tibia (approx dose 4 Gy) and 9 (42 8%) in the vertebral column (approx dose 2 4 Gy) In addition one tumour was found in the sternum (approx dose 1 5 Gy)

In the lowest dose group the osteosarcoma incidence was the same 10 per cent in both sexes In these groups 3 of 10 tumours were found in femur + tibia (approx dose 2 Gy) 4 in the spine (approx

Table 4

Site of malignant bone tumours

Site	Injected amount of ^{140}Ba kBq (μCi)/g b w								Total
	55 5 (1 5)		37 (1 0)		18 5 (0 5)		0 0		
	Male n=50	Female n=48	Male n=49	Female n=48	Male n=49	Female n=49	Male n=70	Female n=70	
Long bones									
Femur	1	2	1	1	–	2	–	–	7
Tibia	–	3		–	–	–	–	–	3
Humerus	–	2	–	1	–	–	–	–	3
Radius	–	–	–	–	1	–	–	–	1
Femur tibia	1	6	–	5	1	–	–	–	13
	2	13	1	7	2	2	0	0	27
Pelvic bones	1	4	–	1	1	–	–	–	7
Spine									
Cervical	–	–	1	–	–	–	–	–	1
Thoracic	2	–	1	2	1	–	–		6
Lumbar	1	3	1	3	1	1	–	1	11
Sacrum	1	1	–	1	–	1	–	–	4
Coccyx	1	–	–	–	–	–	–	–	1
	5	4	3	6	2	2	0	1	23
[illegible]									
[illegible]	1	–	1	–	–	–	–	–	2
[illegible]	1	–	–	–	–	–	–	–	1
[illegible]	–	1		–	–	–	–	–	2
[illegible]	–	–	–	1	–	1	–	–	2
Total	10	22	5	16	5	5	0	1	64

dose 1 2 Gy) and one in the sternum (approx dose 0 7 Gy)

The experimental data indicate that in the strain of mice used the vertebrae might be somewhat more inclined to develop osteosarcomas per unit dose than the long tubular bones In the female control material the osteosarcoma rate was 1 4 per cent The higher incidence of osteosarcomas among females agrees well with previous findings for ^{90}Sr in CBA mice (NILSSON 1962 1967) With reservation for strain differences between C57 Bl and CBA mice it seems to be indicated that ^{140}Ba per dose unit will produce bone tumours more effectively than ^{90}Sr

In previous experiments (NILSSON 1973) female CBA mice developed osteosarcomas only in 6 per cent at a dose level of 1 85 kBq (0 05 μCi) ^{90}Sr/g b w and in 15 per cent when the dose was doubled The majority of the tumours were sited in the femur and the irradiation dose calculated was 33 and [illegible] Gy respectively In rats however STRELTSOVA & MOSKALEV (1961) found that osteosarcomas could be induced by ^{90}Sr in a dose of 6 3 Gy [illegible]

Table 5

Calculated approximate radiation dose in 3 different bones of mice given ^{140}Ba at different dose levels

Bone	Calculated dose (Gy) Inj amount of ^{140}Ba kBq/g b w		
	55 5	37 0	18 5
Femur	6 0	4 0	2 0
Lumbar vertebra	3 6	2 4	1 2
Sternum	2 2	1 5	0 7

he present series 2 osteosarcomas were found in he femur 2 in the lumbar spine and one in the sternum at doses of 2 1 2 and 0 7 Gy respectively However this comparison should be taken with much reservation since the control group of female mice had a spontaneous frequency of osteosarcomas of 1 4 per cent and in addition one osteoma This is a very high figure as compared with that of the CBA mice If the whole material is taken into consideration 58 mice of 293 have developed osteosarcomas after doses not exceeding 6 Gy This may indicate that the continuous irradiation from long lived nuclides to a considerable extent may be wasted i e delivered after the onset of autonomy The surplus irradiation may however play a role or the multiplicity of tumours This should be valid or ^{90}Sr through indications that the multicentric genesis of tumours as well as the tumour latency time are functions of time and the dose accumulated NILSSON 1970) However the present results are too vague to allow a general validity of this statement also for ^{140}Ba

As regards the location of the tumours in various bones no definite difference related to the dose was discernible which was reported to be the case with ^{90}Sr (NILSSON 1970) The reason for this seems to be that high doses of ^{90}Sr will continuously depress or kill the cells at sites where tumours most likely will appear when optimum cancerogenic doses are used Initially ^{140}Ba will give a high dose to the most exposed sites but due to its short half life surplus irradiation will not interfere with reparative and proliferative events or malignant transformations of cells

In the present series rather a decreased incidence was found in contrast to the increased rate of LR tumours reported by STRELTSOVA & MOSKALEV This discrepancy seems largely to be explained by the very high spontaneous frequency of LR tumours in the present strain of C_{57}Bl mice

SUMMARY

C_{57}Bl mice of both sexes were given intraperitoneal injections of ^{140}Ba nitrate at 3 different dose levels (55 5 37 0 and 18 5 kBq/g b w) Osteosarcomas were found in all groups and the osteoblastic type was the most common In the middle and the highest dose groups female mice developed tumours more frequently than the males The irradiation doses were calculated to the femur lumbar vertebrae and sternum and turned out to vary between 6 and 0 7 Gy

ACKNOWLEDGEMENT

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INFLUENCE OF THYROID HORMONES ON ERYTHROPOIESIS AND RADIATION RESISTANCE IN C 57 BLACK MICE

J MIKESKA M POSPIŠIL J NETIKOVA and B HOŠEK

The relationship between the activity of ...mopoietic tissue and its sensitivity to irradiation ...s been discussed in several reports Animals ...ose erythropoiesis had been stimulated by ...posure to hypoxia (TRIBUKAIT & FORSSBERG ...4 FOGH 1971) by bleeding (SMITH & WILLARD ...9) or by injection of penylhydrazine (SMITH & ...KINLEY 1972) showed for a certain period after ...rds higher resistance to radiation It is presumed ...t in these situations the increased erythropoiesis associated with an increase in the number of ...mopoietic stem cells thus facilitating faster ...overy from postirradiation marrow depression ...viously the possibility of stimulating haemo-...iesis and the resistance to radiation of an organ ...n by the application of thyroid hormones before ...adiation was indicated in reports by POSPISIL et ...l (1974) POSPISIL & NETIKOVÁ (1974) and ...SPIŠIL et coll (1975) The practical possibilities of ... use of hormone treatment to influence the resist...ce of an organism led to an extension of the ...periments and further analysis of the mechanisms ... these relationships The results now published ...al with the effect of thyroid hormones admini...rated during 10 days on the resistance of C 57Bl ...ice An attempt is also made to correlate the ...ologic effect obtained with the dynamics of in...encing certain metabolic and haemopoietic func...ons

Methods

Experimental animals and treatment procedure ...ale mice of the inbred strain C ... 10 10 to 12 weeks old with an average body weight of 25 to 30 g were used They were caged in groups of 20 animals Tap water and basal standard diet supplemented with dried pulverized thyroid gland (Thyreoidin Spofa) were given ad libitum Thyroid gland was incorporated into the pellets by food—drug tritura tion at a level of 6 mg/g of food (0 6%) and adminis tered to the mice for a period of 10 days Body weights of the animals were recorded and group food intakes were assessed according to differences

Irradiation The mice were irradiated with a TUR apparatus operated at 180 kV 15 mA filtration 0 5 mm Cu and 0 5 mm Al dose rate 0 46 Gy/min Single whole body doses were applied in the morning hours

Metabolic rate The oxygen consumption was measured individually using an automatic gas analyzer operating on an open circuit principle (HOŠEK & CHLUMECKY 1967) During measurement the mouse was kept in a small chamber 0 5 l in volume ventilated with atmospheric air and main tained at 23 C The data of oxygen consumption given in the results are means of the values obtained between the 31st and 60th minute of the test and are expressed as ml/g of body weight/hour

The amount of iodine bound to proteins of the blood serum was determined according to the method of TŮMA (1975) Blood samples were ob tained by withdrawal from the axillary veins of ether anesthetized mice

Peripheral and bone marrow cell counts Pe ripheral red and white blood cells and nucleated

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elements of femoral bone marrow were estimated by means of a Coulter Counter The number of mobilized granulocytes was determined 8 hours after intraperitoneal injection of endotoxin (Lipo polysaccharide B S typhosa Difco) in amounts of 20 μg/mouse (SMITH et coll 1961) Granulocytes and lymphocytes were calculated on the basis of blood smear differentiation Blood samples were drawn from a fine incision in the tail vein Bone marrow of femoral diaphyses was washed with isologous serum and suspended

⁵⁹Fe incorporation ⁵⁹Fe citrate (Rotop GDR) diluted with saline was injected intraperitoneally in the amount of 7 4×10⁴ Bq After 6 hours the animals were killed by cervical dislocation the spleens were excised and weighed Extraction of heme iron from the spleen was carried out by means of the method of VACHA et coll (1978) with the aid of acid ethyl acetate For measuring of the iron uptake into peripheral red cells the intraperitoneal application of 1 85×10⁴ Bq of ⁵⁹Fe citrate and 24 hour interval of incorporation was used Blood was obtained by severing the vascular bundle deep in the axilla The activities of the heme extract and red cells were measured by means of the Nuclear Chicago Automatic Gamma Well Counting System and expressed as a percentage of injected doses

The red blood cell volume was measured by the ⁵⁹Fe labelled red cell dilution method (VACHA 1975) ⁵⁹Fe labelled red cells were obtained from donor mice injected intraperitoneally with 18 5×10⁴ Bq of ⁵⁹Fe citrate 4 days before and applied intravenously to the experimental animals Blood was drawn from the vascular bundle in the axilla 15 min later and its activity measured Haematocrits were determined using the standard semi micro method

Repopulation assays The principles of the methods of HODGSON (1962) and HELLMAN et coll (1969) were employed Bone marrow pooled from femurs of thyroid treated and control animals was suspended in Hanks solution at ice temperature After counting the number of nucleated cells the suspension was adjusted to the desired concentration (10⁶ cells) and injected into normal syngeneic recipients via the tail vein within 3 hours after their irradiation with 6 7 Gy Nine days later erythropoiesis repopulating ability and granulocyte repopulating ability were determined by measuring either the ⁵⁹Fe incorporation into peripheral red blood cells or peripheral granulocyte response to endotoxin as described

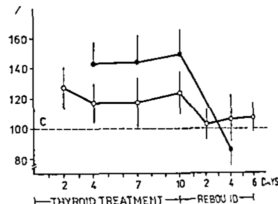


Fig 1 Oxygen consumption (O) and protein bound iodine level in serum (●) in the period of thyroid treatment and rebound The results are expressed as percentages of the control untreated group (C) 6 to 12 animals per group Difference from controls × p<0 05 ×× p<0 01

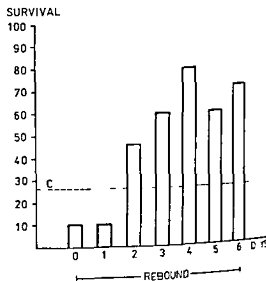


Fig 2 Percentage of 30-day survival after 7 3 Gy given on different days of the rebound after thyroid treatment (C-untreated controls) Ten to 20 animals per group χ^2=?5 ? 6 degrees of freedom p<0 001

Statistics The Student s t test and the chi square test were used The values given in the data represent the mean ± standard error

Results

The body weight of the animals during the 10-day administration of thyroid gland did not differ from that of controls The average food consumption increased gradually in the experimental animals and on the tenth day was about 50 per cent higher than for the controls After the discontinuation of the thyroid administration in the rebound period it gradually decreased to the level of the controls The

Table 1

Erythrocyte lymphocyte and granulocyte (before and after endotoxin) counts per mm³ of venous blood determined 14 days after sublethal irradiation with 4.8 Gy Irradiation of the mice pretreated with thyroid was performed on various days of the rebound 10 to 20 animals per group

	Erythrocytes (×10⁶)	Lymphocytes (×10³)	Granulocytes (×10³)	
			Before endotoxin	After endotoxin
Control	7 39±0 17	1 59±0 09	0 90±0 07	4 00±0 39
Day of rebound				
0	7 65±0 24	1 49±0 11	0 81±0 07	3 34±0 55
1	8 23±0 19	2 06±0 23	1 06±0 11	5 17±0 48
2	8 50±0 18	1 29±0 16	1 90±0 31	11 39±[illegible] 40
3	9 15±0 18	[illegible] 84±0 [illegible]4	1 76±0 [illegible]8	9 66±1 02
4	8 42±0 16	2 45±0 38	1 65±0 16 *	9 68±1 64
5	8 33±0 15	2 42±0 26*	1 50±0 17	5 99±0 95

Difference from controls p<0 05 * p<0 01

Table 2

⁵⁹Fe incorporation into heme of spleen (in percentages of injected activity) wet spleen weights and nucleated cells per femur in control mice and in animals from various intervals of thyroid treatment and rebound 6 to 11 animals per group

	⁵⁹Fe/spleen heme (per cent)	Spleen weight (mg)	Nucleated cells (×10⁷)/femur
Control	1 04±0 17	7[illegible] 9±2 6	1 38±0 04
Day of thyroid treatment			
7	1 77±0 29	108 4±6 0	1 45±0 04
10	4 56±0 62	150 9±6 7*	1 68±0 09
Day of rebound			
1	5 24±0 66	149 6±5 3	–
2	4 79±0 29	155 1±[illegible] 9	1 62±0 08
3	5 71±0 50	141 1±8 2	–
4	2 27±0 48	96 7±5 6*	1 84±0 04
5	2 30±0 45	98 6±5 4	1 84±0 06
6	1 38±0 20	84 5±4 0	–

Difference from controls p<0 05 p<0 01

consumption of oxygen increased in the experimental group 2 days after the start of thyroid administration by about 30 per cent maintaining these values during the remainder of the 10-day treatment period and falling to the level of the controls during rebound (Fig 1) The level of protein bound iodine in the serum during thyroid administration is an indirect indication of an increased level of thyroid hormones in the blood and has a parallel course to the oxygen consumption (Fig 1)

Fig 2 gives the results of experiments comparing the survival of animals irradiated with a dose of 7 3 Gy administered at various times during the rebound period after the thyroid administration The results show a slight reduction of the resistance to radiation at the time of the cessation of the administration of thyroid hormones and a subsequent increase of the resistance peaking on the fourth day of the rebound period The experiments to ascertain the level of red blood cells lymphocytes and granulocytes (counts

at rest and after endotoxin mobilization) 14 days after sublethal irradiation of animals with 4 8 Gy indicate an increase in the regeneration potential of haemopoiesis among animals irradiated during the rebound period (Table 1)

The determination of the volume of red blood cells in animals on the tenth day of treatment with thyroid gland showed a significant increase in the values ($p<0\ 01$) with $0\ 91\pm0\ 05$ ml/mouse ($n=10$) as opposed to the control value of $0\ 64\pm0\ 02$ ml/mouse ($n=10$) thus demonstrating high activation of erythropoiesis by the thyroid hormones The insignificant differences in haematocrit values ([illegible]$\pm1\ 0\%$ for experimental animals $48\ 8\pm1\ 0\%$ for control animals) indicate a parallel increase in the plasma volume of treated animals

The dynamics of the activation of erythropoiesis in the phase of hormonal treatment and in the rebound period were analysed by the determination of ^{59}Fe incorporation into the heme fraction of the spleen The results (Table 2) indicate a strong increase in spleen erythropoiesis during thyroid hormone treatment which is maintained during the first 3 days of the rebound period and then a sharp decline to control values on the fourth day of rebound Spleen weight behaves parallelly

The characterization of haemopoietic progenitors in the bone marrow of animals which had undergone thyroid gland treatment was carried out by means of repopulation assays The erythropoiesis repopulating ability and granulocyte repopulating ability of bone marrow cells of animals treated with thyroid hormones was measured after their transplantation to normal irradiated recipients The results appear in Fig 3 Towards the end of the period of thyroid gland administration a slight and non significant decrease occurred in the values of both repopulation assays During the rebound period the repopulating ability of bone marrow increased and reached statistical significance in both tests on day 4 5 and 8 The results of repopulation assays relate to a similar quantity of injected nucleated cells (10^6) The increased number of nucleated cells in the femurs of animals treated with thyroid gland (Table 2) determined in the period of the fourth and fifth days of rebound indicates that the results of erythropoiesis and granulocyte repopulating ability relate to an absolute and not merely a relative increase in the repopulating ability of the bone marrow during this period

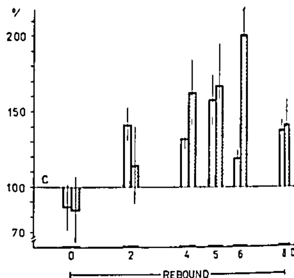


Fig 3 Erythropoiesis (□) and granulocyte (■) repopulating a[...]ty of the bone marrow on various days of the rebound [...] thyroid treatment expressed as percentages of the values f[...] untreated controls (C) Five to 15 donors as recipients per gr[...] Difference from controls × $p<0\ 05$ ×× $p<0\ 01$

Discussion

Previously above all the radiation sensitizing eff[...] of thyroid hormones has been demonstra[...] (BLOUNT & SMITH 1949 SMITH & SMITH 1951) [...] increased metabolic rate at the time of radiat[...] exposure is regarded as an unfavourable fact[...] aggravating primary radiation lesion and decreas[...] the radiation resistance of experimental animals [...] similar mechanism may be involved in the pres[...] experiments on the irradiation of animals immedia[...]ly after the discontinuation of thyroid hormones a[...]ministration An increase in resistance to radiatio[...] observed in the later period after the cessation [...] thyroid gland administration coincides with the a[...]justment of the metabolic state In view of the fa[...] that the consumption of oxygen does not fall dur[...] this period to below the control level the resu[...] cannot be considered as a protective effect [...] hypometabolic states (BRAUN et coll 1964)

Hypermetabolic conditions including hyp[...]thyroidism lead to higher demands on oxyg[...] supply to tissues activate erythropoietin regulati[...] and stimulate erythropoiesis (KRANTZ & JACOBS[...] 1970) The increased erythropoiesis can be a[...]sociated with an increase in the total number of s[...] cells and thus create more favourable conditions f[...] the repopulation of the haemopoietic system af[...] irradiation (SMITH & WILLARD FOGH) A sim[...] mechanism may be at work in the present exper[...]ments and the effect of the thyroid pretreatme[...]

may be similar to that obtained by the application of other factors stimulating erythropoiesis such as long term hypoxia (TRIBUKAIT & FORSSBERG ...IBUKAIT 1966 1969) However in the case of the ...fect of hormones the more complex stimulatory ...chanism must be taken into account In addition ...indirect calorigenic and erythropoietin activating ...fects the hormones of the thyroid may also have a ...rect stimulatory effect on the processes of cell ...oliferation and differentiation (MALGOR et coll ...75 GOLDE et coll 1977)

The experiments now presented support the idea ...at the activation of erythropoiesis and stem ...ll populations is important for influencing the ...diation resistance of an organism by the previous ...ministration of thyroid gland Erythropoiesis in ...e spleen which is a particulary reactive organ of ...tramedullary erythropoiesis in mice (BOZZINI et ...ll 1970) demonstrates the high activity during the ...ministration of thyroid hormones its continuation ...ring the period of rebound and its rapid decline ...the fourth day of rebound The continuation of ...creased values of heme formation in the spleen ...ring the first days of rebound can be explained by ...e accumulation of erythroid precursors due to ...eir lower transformation output into mature cell ...rms after the reduction of demand for red cell ...roduction Similarly the increase of the repopulat...ng ability of the bone marrow cells in the rebound ...eriod after thyroid pretreatment may be explained ...y a decreased output of cells from the undifferen...ated or less differentiated stem cell compartments

Transplantation methods evaluating erythropoie...s and granulocyte repopulating ability of the bone ...arrow cells measure a broader part of the con...um of the stem cell compartment including both ...luripotent and unipotent stem cells committed to a ...pecific differentiated pathway (GOODMAN et coll ...977) It is therefore not possible to determine on ...his basis the type of stem cell population affected ...y the use of the particular experimental measures ...It is however possible to point out the results of ...ACEK et coll (1978) showing a rapid increase in the ...ool and proliferation of pluripotent stem cells (CFU ...) in thyroid treated mice

Th... idea that an increased number of haemopoiet...c stem cells or a higher repopulating ability of bone ...arrow cells at the time of radiation risk is an impor...ant factor may not however be an accurate indica...ion of the real situation It is essential to take into ...ccount other possible mechanisms such as e g changes in the radiation sensitivity of the stem cells Unpublished results from another strain of mice did not indicate a modified radiation sensitivity of haemopoietic stem cells in thyroid pretreated animals BERAN & TRIBUKAIT (1972) showed that the application of hypoxia to animals before irradiation does not change the degree of survival after irradiation of CFU S Similarly SMITH & MCKINLEY did not find any change in the sensitivity of stem cells in vivo after stimulation of erythropoiesis with phenylhydrazine BERAN & TRIBUKAIT (1974) further showed that an improved growth of stem cells (CFU S) in mice after hypoxia can also be conditioned by changes in the physiologic microenvironment The role of similar influences in the present experimental model using hormones of the thyroid gland remains to be analysed

SUMMARY

Oral administration of dried thyroid gland to C_3 Bl adult male mice in a dose of 0 6 g per 100 g diet during 10 days increased the metabolic rate and stimulated the erythropoiesis Three to six days after thyroid pretreatment the radiation resistance of the mice increased as revealed by their 30-day survival and a higher recovery of peripheral blood cell counts The period of increased resistance to radiation was correlated with the receding of hypermetabolic effects and with the increase of repopulating abilities of the bone marrow cells

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ELECTRON AND PHOTON BEAMS FROM A 50 MeV RACETRACK MICROTRON

A BRAHME T KRAEPELIEN and H SVENSSON

Accelerator produced high energy electron and ...ton beams have been used for radiation therapy ...ce the early fifties The radiation modalities of ...st frequent use today are of low and medium ...rgy from a few MeV up to about 20 MeV This is ... case even though for many target locations ...ter dose distributions are obtained at considera... increased beam energies The development and ... of high energy radiation modalities with energies ... to about 50 MeV have been limited by a number ...actors

...ne of the most important reasons is the technical ...blems of obtaining radiation beams of high qual... at these high energies In the case of electron ...ms the advantageous steep dose fall-off behind ... therapeutic range is largely lost in particular ... ordinary scattering foils are used to flatten the ...m For the photon beams the problem is instead ... the very thick flattening filters needed will de...ase the effective photon energy so that only a ...all improvement in beam penetration is obtained ... increasing the beam energy

...mpediments of almost equal significance have ...n the absence of a small compact and light weight ...elera or of sufficient output and low cost The ...atrons for example have been restrained by their ...vy weight low dose rate and often poor dose ...ributions at least for the electron beams Similar ... the high energy linear accelerators have been ...rained mainly by their large dimensions or high ...wer requirements and costs but also by the beam flattening problems mentioned However the race track microtron which is the most recently developed electron accelerator in this energy range (WERNHOLM et coll 1974) is not restricted in these respects due to its small size (1 5 m×0 5 m×0 4 m) light weight (1 500 kg) and high beam current (20 μA at 50 MeV)

Some new methods to improve the usefulness of high energy photon and electron beams based on such an accelerator are therefore discussed This is done not only in terms of the spatial distribution of absorbed dose but also in terms of the relative biologic effectiveness considering different fractionation patterns and grid techniques The proposed new treatment techniques are illustrated by dose distributions measured in the research beam from a 50 MeV racetrack microtron

Accelerator

The working principle of the racetrack microtron (Fig 1) was suggested by SCHWINGER (quoted by SCHIFF 1946) only a few years after the principle of the conventional microtron was presented by VEKSLER (1944) The fundamental improvements gained by splitting the magnet of a conventional microtron in two equal halves are threefold

(1) A new degree of freedom is introduced allowing the use of much higher magnetic fields resulting

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in a very compact accelerator and continuously variable electron energy

(2) The field free region between the two magnet halves gives more space for the acceleration section allowing larger energy gain per revolution than is possible with a single resonator cavity

(3) The focusing of the accelerated electrons can likewise be improved in the gap between the magnets by quadrupoles or special design of the semicircular magnets (FROELICH & BRANNEN 1967 BABIC & SEDLACEK 1967)

The development and properties of the racetrack microtron were discussed in detail by WIIK & WILSON (1970) and the particular machine used in the present investigation was described in more detail by WERNHOLM et coll The use of an 18 or 25 MeV racetrack microtron for radiation therapy was discussed previously by MACDONALD & FROELICH (1974)

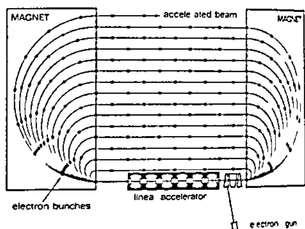


Fig 1 Cross section through the orbital plane of the racetrack microtron used Electrons are accelerated up to 15 times by the short linear accelerator section with a 3 3 MeV energy gain per revolution giving a maximum energy of about 50 MeV Electrons are injected by a small deflection magnet in front of the linear accelerator

Fundamental considerations

Electron therapy The design of broad uniform electron beams of high energies and large therapeutic ranges has been discussed extensively in the literature The basic problems involved can be subdivided into two parts The first concerns the problems of obtaining flattened electron beams with uniform distribution and the second concerns how therapeutic ranges are obtained

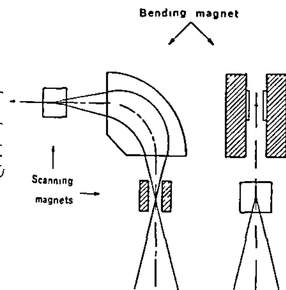
The most common method of beam flattening is by using a single scattering foil This is not satisfactory except possibly for small field sizes and energies below about 10 MeV since the thick scattering foils otherwise needed degrade the electron beam quality considerably (BRAHME & SVENSSON 1976a 1979) The dual scattering foil technique is about one order of magnitude better with regard to the amount of material in the beam and will thus give high quality electron beams at energies up to about 20 MeV (BRAHME 1977) However above this energy the beam degradation again becomes considerable At the highest energies from about 25 MeV to 50 MeV the electromagnetic systems using defocusing or scanning magnets or quadrupoles (HSIEN & UHLMANN 1956 AUCOUTURIER et coll 1970) are the only possibilities for obtaining high quality beams

Among the methods used to obtain an increased therapeutic range are linearly moving deflection magnets (ROZENFELD et coll 1969) These change the normal quadratic dose decrease with distance which is obtained with a small isotropic radiation source to a linear dose decrease obtained with a line source This will increase the therapeutic depth of the beam as the dose fall-off of the depth dose curve will be decreased

A method which uses a similar principle is the small angle pendular technique practised with the fan beams of some betatrons (RASSOW 1970) However similar results can be obtained with most treatment units simply by applying a multitude of parallel or convergent beams In the limit of a continuum of displaced fields (arc or moving beam therapy) the geometric dose decrease can even be transformed to an increase

From this point of view it should be sufficient for most practical purposes to use a scanning system where the effective radiation source is small In Fig 2 such a system is illustrated which also is capable of scanning the photon beam It consists of two scanning dipole magnets each deflecting the beam in one of the orthogonal planes and with a bending magnet in between By using the beam optical properties of the bending magnet the first scanning dipole can be placed in front of the bending magnet with the image of its scanning centre near the middle of the second dipole which is scanning in the opposite plane In this way a very compact deflection system is obtained with the position of the effective radiation source coinciding for the two orthogonal planes

A number of advantages are inherent in such a beam scanning system not least from a beam geometric point of view It implies a well defined



ig. 7 Design of proposed canning system for the electron and oton beams from an electron accelerator (two orthogonal sec ons) The photon beam is scanned by placing the target im ately below the last scanning magnet

ource to surface distance (SSD) and the fact that e emergent beam for most practical purposes can e described as a point isotropic beam with the same uence and energy distribution in all directions In ddition the distortion of the elementary beam by he simple scanning dipoles will be considerably less han if a more complex quadrupole is used to scan he beam in two planes

Photon therapy The scanning system shown in ig 7 is of special interest since the second dipole an be made very strong and short so that a remsstrahlung target can be placed close to the ffective electron source and thus convert the canned electron beam to a scanned photon beam This is of considerable importance not only as it will make broad uniform photon beams available at any photon energy without the need of thick flattening filters but also because the photon spectrum will be mproved when the degrading flattening filter is re moved and the hard forward peaked photon spec trum is distributed uniformly over the entire radia tion field

The conventional methods of obtaining broad uni form photon beams have been discussed extensive ly in the literature Two principle designs have been suggested either using a low atomic number target and flattening filter to produce a beam of the highest possible mean photon energy and penetration on the central axis of the beam (PODGORSAK et coll 1974) or using a thin high atomic number target with an electron stop and a composite flattening filter to produce a photon beam of high uniformity at all depths and field sizes due to simultaneous uniformi ty in photon fluence and mean photon energy (BRAHME & SVENSSON 1976b) The first method will produce very penetrative narrow beams at the cost of a variation in uniformity with depth for broad beams The second method will instead produce uniform beams practically independent of field size and depth but at the cost of a slightly reduced penetration at small field sizes

The suggested scanned photon beam combines the advantages of the photon beam designs men tioned but avoids their respective disadvantages Furthermore the scanned photon beam will also increase the available dose rate since a flattening filter which often absorbs as much as 50 to 80 per cent of the photon fluence is no longer needed As a consequence the radiation shielding problems out side the field will be decreased by a factor 2 to 5 and so will the neutron production in the flattening filter and collimators This last problem which causes much debate today particularly in the USA (cf NBS 1979) can thus be reduced considerably by using a scanned photon beam

Radiation biology The production of broad un iform radiation beams by scanning a narrow elemen tary beam has a number of consequences of biologic nature which may become of clinical value

The radiation beam from most accelerators con sists of short intense pulses of a few microseconds duration at repetition rates of about a hundred pulses per second Therefore when the elementary beam is of small cross section each intense pulse may be placed in an orthogonal or hexagonal pat tern to obtain dose distributions similar to those used in grid or sieve therapy

GRIEM et coll (1969) reported absence of skin reaction using a pulsed scanning electron beam for radiation therapy Their scanning system gave an almost uniform dose distribution in the superficial skin layer therefore a sieve effect was unlikely to be the explanation for the absence of reaction They also showed in survival curves for Chinese hamster cells irradiated with two different beam currents during the pulses but with the same total absorbed doses to the cells that the high dose rate gave the smallest biologic effect A possible explanation for this effect could be a depletion of intracellular oxygen However a very large dose per pulse is necessary to give this effect (EPP et coll 1968)

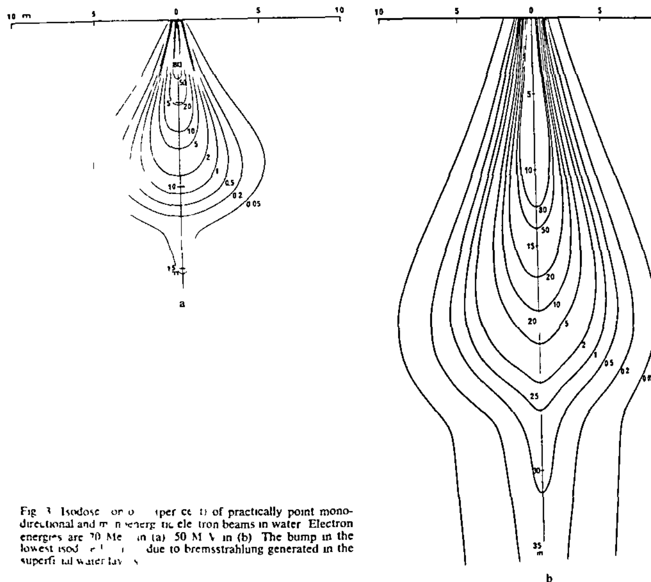


Fig 3 Isodose or o (per ce t) of practically point mono-directional and m n energ tic ele tron beams in water Electron energies are 20 Me in (a) 50 M V in (b) The bump in the lowest isod e l i due to bremsstrahlung generated in the superfi ial water lay s

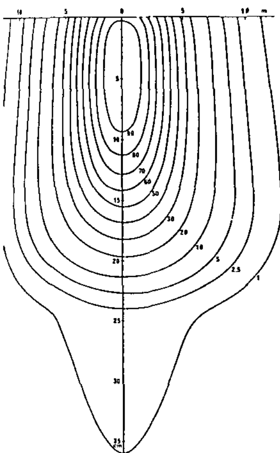
making this explanation unlikely No conclusive explanation has yet been given

An effect working in the opposite direction should be expected at short pulses of very high dose rate as a result of instantaneously overlapping electron tracks This should result in an increased RBE at extremely high dose rates due to increase in effective LET The pulse length needed for such effects to be important is about 50 ns and the dose rate in the pulse should be above 10^8 Gy/s which is a regime marginally accessible with a scanning beam system

The sieve therapy was much analysed and used in the days of conventional roentgen therapy in order to decrease skin reactions e g JOLLES (1953) BAUM (1958) but is also stated to be justified for high energy photon beams (BECKER et coll 1958 ALIEV 1976) With electron radiation below about 20 MeV there seems to be no reason for grids as the

low energy electrons are used to obtain a u beam in a target volume at a small depth Ho at higher electron energies the target volume often situated at a large depth and it may be de to save the normal tissue at small depths technique could in such cases be very con as a grid effect can be obtained at small but a complete uniform irradiation at larger With a grid technique fairly massive doses co given to normal tissue ALIEV used on some p (in his series of 251 patients) more than 220 G out any severe reaction No clinical or exper investigation for high energy electrons above 20 MeV is available

Combination effects may be gained with a s scanning system Thus the low skin reactio high pulse and dose rate reported by GRIEM may be combined with a favourable grid tec The reduction in the response of normal



4 Isodose contours (per cent) of a 45 6 MeV Gaussian ectron beam produced by a narrow pencil beam transported rough vacuum window dose monitor and 1 m of air Resultant ot mean square radius is 45 5 mm

ompared with conventional therapy at a low dose te with uniform irradiation of the superficial tis e is impossible to estimate from presently avail al literature Many of the experimental results ported in the following have been obtained in in stigations of the technical physical and biologic ntials of a scanning beam system for radiation eatments

Electron absorbed dose distributions

Pencil beams Due to their importance for the anned and grid beam techniques dose distribu ns from almost point monodirectional and noenergetic electron beams have been measured e isodose curves for such pencil beams of 20 and MeV electrons are presented in Fig 3 The dose tributions were measured in a 40 cm×40 cm×40 water phantom with a 0 1 mm mylar window ed immediately after the electron beam left the uum system through a 0 08 mm aluminium foil The root mean square diameter of the electron beam was less than 5 mm with practically no electrons outside a diameter of 10 mm The angular di vergence of the beam due to the emittance of the accelerator the focusing of a weak quadrupole lens and additional scattering in the vacuum window was only a few degrees It was essential that the elec tron beam was not collimated by a narrow material aperture as that would generate excessive bremsstrahlung contamination which would disturb the measurements The energy spread of the beam was less than 0 2 MeV at the full width at half the maximum electron fluence

The absorbed dose distribution was determined by a small silicon diode of 2 mm×2 mm cross sec tional area The dose distributions were checked with ferrous sulphate measurements in a 40 MeV electron beam The agreement was better than 1 per cent over the entire range of measurement from a depth of 2 to 22 cm in good agreement with previous diode measurements at lower energies

The absorbed dose in a pencil beam decreases very rapidly with depth as the beam is spread out in the water (Fig 3) The isodose curves have there fore somewhat arbitrarily been normalized to 100 per cent at a depth of about 2 and 12 cm respective ly

The characteristic pear shape of the isodose curves is fundamentally due to the accumulative multiple scattering interactions of the electrons in consecutive water layers in combination with their decreasing energy It is of interest to see how the pear shape at the lowest isodose levels is influenced by the strongly forward peaked bremsstrahlung generated in the first part of the electron tracks It is also observed that the photon tail is more marked at the higher energies as should be expected

Of particular interest is that the width of the dose distribution does not vary much with the electron energy This effect is caused by the high scattering power and low penetration depth at low energies and the increasing penetration but simultaneously de creasing scattering power with increasing energies More exactly the range is directly proportional to the energy and the scattering power inversely pro portional to the energy squared Since the lateral de flection is approximately proportional to the product of range and root mean square deflection angle it will thus only have a very weak energy dependence

This general result indicates that in practice the same grid spacing could be used for sieve therapy

independent of electron energy at least when a uniform absorbed dose is desired at the treatment depth but not at the surface

Gaussian beams A point monodirectional or pencil beam can only be used for radiation therapy if the vacuum window is thin and placed close to the patient Even if such a system is in clinical use (ROZENFELD et coll) it is not a very practical one for routine radiation therapy The beam normally has to be transported some distance through air after having penetrated the vacuum window and has also to pass a transmission monitor and mirror for light field indication All these components will spread the electrons so that the electron beam reaching the patient will necessarily have a certain width Fig 4 shows the lateral isodose distribution of such a 46 MeV electron beam which has passed through the vacuum window (0 08 mm aluminium) 1 m of air and 3 monitor foils (0 03 mm molybdenum) This electron beam can for most practical purposes be described by a Gaussian radial distribution From a comparison with Fig 3 it is seen that the characteristic pear shape of the pencil beam is almost lost largely due to the influence of the bell shaped lateral dose decrease

From these results it can be concluded that in order to obtain a strong good effect (large ratio between peak dose and background dose) the amount of material in the beam should be minimized

Broad beams Three basically different techniques have been used in the present experiments to obtain the central axis depth dose distributions for broad electron beams

The first method used was a thin scattering foil combined with a very long source to surface distance in order to produce broad almost parallel and monenergetic electron beams The results appear in Fig 5 for intervals of 10 MeV from 10 to 50 MeV at an SSD of 200 cm and a scattering foil of 0 1 mm lead When the appropriate corrections are made for the collisional energy losses in the vacuum window scattering foil and air the most probable accelerator energy determined from the practical range of these curves agree within 0 3 MeV with the accelerator energy obtained from the magnetic field of the accelerator The energy range relation used was

$$(E_p)_0 = C_1 + C_2 R_p + C_3 R_p^2 \quad (1)$$

or

$$R_p = C_4 + C_5 (E_p)_0 + C_6 (E_p)_0^2$$

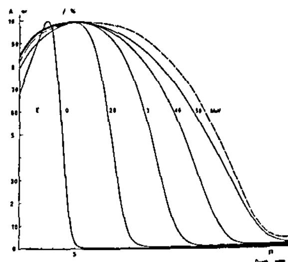


Fig 5 Electron central axis depth dose curves produced by a scattering foil of 0 1 mm lead at a SSD of 200 cm (—) Dashed curve obtained by dual foil technique with a primary foil of 0 1 mm gold and a shaped secondary foil of 1 8 mm aluminium

where

$C_1 = 0\ 22$ MeV	$C_4 = -0\ 11$ cm
$C_2 = 1\ 98$ MeV cm^{-1}	$C_5 = 0\ 505$ cm MeV^{-1}
$C_3 = 0\ 0025$ MeV cm^{-2}	$C_6 = -0\ 0003$ cm MeV^{-2}

The discrepancies observed were all within the uncertainty of the magnetic field determination The traditional energy range formula derived by MARKUS for the energy range below 20 MeV (ICRU 1972 NACP 1972) is in error by as much as 2 5 MeV at 50 MeV Relation (1) is now recommended by the NACP (1980) for the energy range 1 to 50 MeV

The small increase in therapeutic range above some 30 MeV even at this fairly large source to surface distance is evident However the beam is far from broad at the highest energies Instead it is practically Gaussian with root mean square diameters of only 16 8 cm at 50 MeV and 20 6 cm at 40 MeV

The second method used was the dual scattering foil technique with which perfectly uniform electron beams can be produced with a minimum of scattering material (BRAHME 1977) The dashed curve in Fig 5 was produced in this way with a primary scattering foil of 0 1 mm gold and a secondary Gaussian shaped foil of aluminium 1 8 mm thick at the centre With this foil combination the beam is perfectly flat out to a diameter of 20 cm A substantial improvement is seen compared with the single scattering foil beam even though the foil thickness was increased considerably In Fig 6 the isodose d

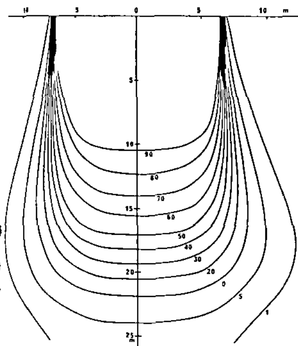


Fig 6 Isodose contours (per cent) corresponding to the dashed depth dose curve in Fig 5 Field size is 14 cm×14 cm and accelerator energy 50 MeV

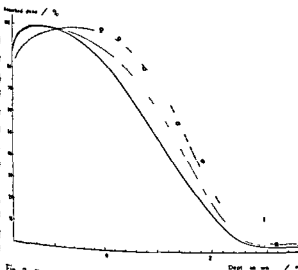


Fig 7 Electron central axis depth dose curves in water at E_0 45 6 MeV Solid line corresponds to Gaussian beam in Fig 4 (SSD 100 cm $\varnothing_{rms}$ 9 1 cm) Dashed curve is obtained by recti linearly scanning the Gaussian beam to produce a plane parallel beam (SSD≈ $\varnothing_{rm}$ ∞) In the dotted curve scanning was point isotropic as would be obtained according to Fig 2 (SSD 100 cm $\varnothing_{rms}$ ∞) Circles correspond to the dashed curve but with a pencil beam similar to Fig 3

tribution of this beam is shown for a 14 cm square field It appears that uniform electron beams of fairly high quality can be obtained with the dual foil technique at intermediate field sizes and a large SSD

The third method used to obtain broad beam dose distributions is based on the integration of narrow beam isodose distributions With this method broad beam data can be obtained by summation of a large number of narrow beams (HARDER 1965) This method could also be described as a mathematical way to simulate the broad beams produced by the rectilinear scanning of a narrow beam

Two different sets of narrow beam data were used for this purpose the pencil beam similar to that in Fig 3 and the Gaussian beam in Fig 4 The integra tion of the pencil beam isodoses was performed by fitting the lateral fluence at each depth to a curve of the type

$$D(r) = \frac{D(o)}{1+ar^b} \tag{2}$$

The central axis absorbed dose distribution in a broad circular beam of a given radius is due to the reciprocity obtained by integrating eq (2) out to the desired beam radius

The result for the pencil beam is shown as circles in Fig 7 for a beam diameter of 15 cm The shape of the depth dose in the surface region could not be determined accurately in this way for the pencil beam since this region was distorted considerably by the finite size of the detector being almost equal to the diameter of the beam For this reason the Gaussian beam data were also used to calculate broad beam dose distributions Due to the quite different lateral dose distribution the lateral profiles were instead approximated by a Gaussian radial dis tribution

$$D(r) = D(o)\exp^{(-r^2/r_0^2)} \tag{3}$$

This gave more accurate broad beam depth dose data since the finite detector size had no influence on the much wider crude beam isodose distribution of Fig 4 The resultant depth dose curve also appears in Fig 7 A fair agreement in curve shape is found between the integrated Gaussian beam (the dashed curve) and the pencil beam but the lesser accuracy of the latter is evident particularly at small depths where the pencil beam is very narrow A few interesting conclusions can nevertheless be drawn from its more accurate dose fall-off section First the range of the pencil beam is slightly longer due to the one meter of air and the monitor foils which were present when the elementary pencil beam distribu tion was measured Secondly the dose gradient is

not so good for the pencil beam curve One possible explanation for this is that the angular spread in the pencil beam of a few degrees is sufficient to reduce the dose gradient (BRAHME & SVENSSON 1978) In the crude beam case the almost plane parallel central part of the beam is mainly used (cf Fig 4)

From the comparison of central axis depth dose distributions in Fig 7 the considerable improvement in depth dose characteristics produced by good uniformity and a long source to surface distance is evident In order to separate these two effects the inverse square law has been included in the integra ... he dotted curve) The difference between the ... and the dotted curves on the other hand are ... is due to the inverse square attenuation of the ... lection fluence which evidently is most responsible fo the loss of therapeutic range at these high energies

However it should be emphasized that both the calculated curves in Fig 7 are somewhat degraded since the Gaussian beam was not strictly parallel Therefore the calculated plane parallel beam contains a small angular spread of a few degrees which probably has decreased it therapeutic range and dose gradient by a small amount (BRAHME & SVENSSON 1978) Th point i otropic beam is somewhat more unce t i n due to the approximate nature of the inverse squ n correction and to the fact that this w is m ide on a b eam which already contained a slight divergence

Photon absorbed dose distributions

Pencil beams Narrow beams of high energy photons are of interest not only for the understanding of the basic interaction processes of photon beams with matter but also for direct application in radiation therapy and surgery of deep seated organs (SARBY 1974) For these purposes it is of great value to know the depth dose and isodose curves in centimeter sized photon beams of very high energy

The isodose curves of a 10 mm diameter photon beam of 46 MeV peak energy and a SSD of 100 cm appear in Fig 8 This beam is obtained from the central part of the unfiltered bremsstrahlung beam produced by an intermediate thickness target of 5 mm tantalum backed up by 25 mm of aluminium for electron absorption It is immediately observed that this high photon energy is of great interest for medical use due to its narrow penumbra and large half value depth of about 19 cm

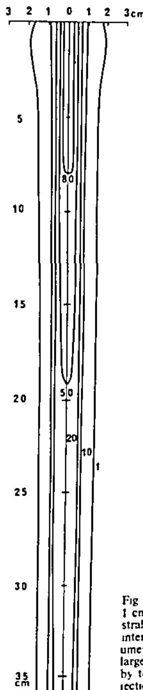


Fig 8 Isodose contours (per cent) of a 1 cm diameter 46 MV non filtered bremsstrahlung beam This type of beam is of interest for small localized target volumes but also for dynamic treatment of larger volumes of more complicated shapes by techniques analogous to the back projection used in a CT scanner

A number of physical properties of the energy deposition of high energy photon beams can be observed Even though the range of the secondary electrons and positrons produced by this 46 MV beam is quite large (almost 10 cm) they deposit nearly all their energy inside a beam with a narrow penumbra region of about 5 mm between the 80 and 20 per cent isodoses The reason for this is that most of the secondary photons and electrons are stro ly forward peaked and thus the beam acts like a continuous line source of high energy electrons (cf Fig 3) which deposit most of their energy close to th

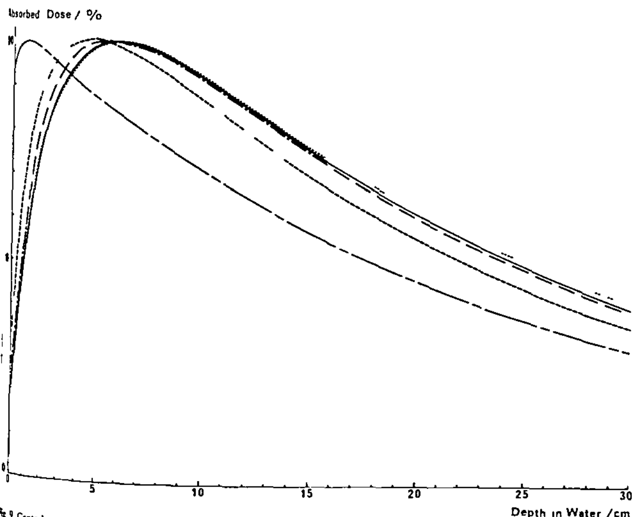


Fig 9 Central axis depth dose curves for elementary non filtered photon beams (46 MV photons) Dashed curve with superficial ...e maximum is the pencil beam of Fig 8 Lateral dose distribution of the other curves appears in Fig 10 Targets 1 05 mm Ta+21 5 cm C (–) 21 5 cm C (—) 1 5 mm Ta+11 cm Al (– –) 9 mm Ta (– – –) 5 mm Ta+2 5 cm Al (Ø 1 cm ——–)

...xis The very small dose build up seen on the central axis depth dose curve in Fig 9 is caused by the quickly established partial equilibrium of photon generated electrons Already after a few cm depth a balance is obtained between those electrons which are lost from the beam by multiple scattering interactions and those generated in the beam by photon interactions The shape of the depth dose curve is except for the build up region similar to the depth dose obtained in a broad beam of 10 MV photons

Elementary photon beams The elementary unfiltered photon beam obtained when a narrow electron beam strikes a target is of fundamental importance for the present experiments since this is the beam which will be scanned to produce broad uniform photon beams The distribution of absorbed dose in elementary photon beams is dependent on the properties of the target At the high energies which presently are of main interest there is only a small variation in dose rate between different target materials (PODGORSAK et coll) However with decreasing atomic number and target thickness the lateral width of the elementary beam will decrease considerably and the high energy photons will more and more be peaked in the forward direction

This is evident in Fig 10 where the lateral dose distributions at dose maximum of the elementary photon beam from a number of different targets are shown In order to simplify extrapolations to different beam energies the lateral variable was chosen as the product of peak photon energy and angular direction from the incident electron beam By using this variable the distributions will have a very small energy dependence as both the photon production angle (m c /E cf the circle in Fig 10) and the root mean square electron scattering angle of the electrons in the target ($(\overline{\theta^2})^{1/2}$) are inversely proportio-

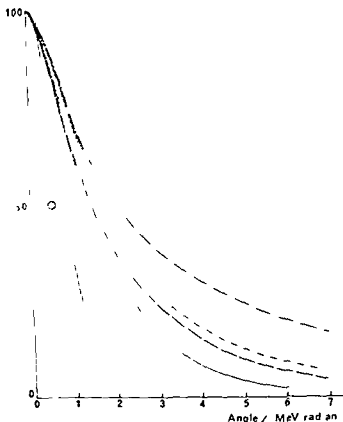


Fig 10

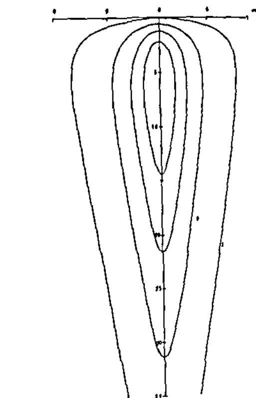


Fig 11a

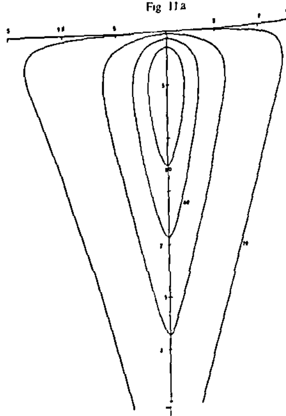


Fig 11b

Fig 10 Angular dose distribution of non filtered bremsstrahlung photons from different targets The circle indicates the opening angle of the pure bremsstrahlung process (m_ec^2/E) Targets 9 mm Ta (— —) 1 5 mm Ta+11 cm Al ( ) 1 05 mm Ta+21 5 cm C (—) 21 5 cm C (- -) 5 cm C (——) 9 mm Be (——) 1 mm Be (---)

Fig 11 Isodose contours curves (per cent) of 46 MV elementary photon beams in water at a target to surface distance of 100 cm For curve a) the target was 21 5 cm of graphite and for curve b) 9 mm of tantalum

nal to the energy of the incident electrons (BRAHME & SVENSSON 1979)

It should be observed that the broadest angular distributions are obtained for thick high atomic number targets where the electron scattering con tribution is largest Similarly the narrowest beam are obtained in thin targets of low atomic numbe where minimum influence of electron multiple sca tering is obtained and the angular spread approache that of the pure bremsstrahlung process (cf the ci

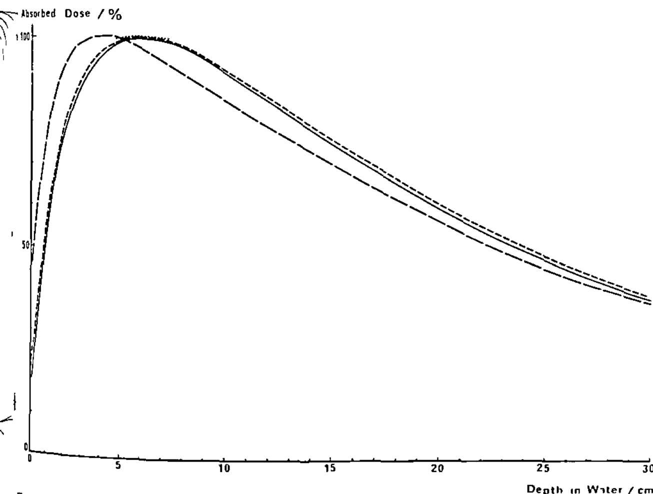


Fig 12 Central axis depth dose curves of 50 MV photons at an SSD of 100 cm Solid line represents non filtered elementary beam Dashed curves are filtered by titanum (4 cm ---) to a cross section of 8 cm and by lead (4 cm ——) to 30 cm respectively Dotted curve obtained by scanning the elementary beam over a circular area of 30 cm cross section

cle in Fig 10) The thin target distributions were obtained with a deflection magnet behind the target to deflect the transmitted electrons out of the useful beam In addition the measurements were made at a large depth in water (30 cm) so that the influence of scattered electrons in the incident beam was minimized

In Fig 9 several examples are shown of the central axis depth dose distribution in elementary photon beams The deepest dose maximum and highest depth dose is obtained for the thin high atomic number target with the carbon electron stop and not for the pure carbon target This somewhat surprising result is due to the narrower width of the pure carbon target beam (cf Figs 10 and 11) which nevertheless has the lowest surface dose

The thick high atomic number target produces with large margin the least penetrative beam even though this beam is more similar to a broad beam The reasons for this are threefold First the radiative stopping power is very high even at low electron energies therefore a considerable amount of low energy bremsstrahlung is generated by the slowing down of the electrons Secondly the very high energy photons from the superficial layers are heavily filtered in the deeper layers in high atomic number targets whereas the low energy photons are filtered more in low atomic number targets Thirdly the high energy photon components are spread over wider angles due to a greater influence of multiple electron scattering in a high atomic number target which again decreases the mean photon energy in the forward direction

The isodose distrubtions of the elementary photon beam from a low and a high atomic number target are shown in Fig 11 at a SSD of 100 cm This is perhaps an even more impressive demonstration of the difference in lateral spread and penetration of the elementary photon beam from different types of targets The low atomic number target was 21 5 cm

of graphite and the high atomic number target was 9 mm of tantalum

Broad photon beams The choice of an elementary non filtered photon beam for generation of broad therapeutic photon beams will greatly depend on the method of beam flattening used If the conventional technique with thick flattening filters is used the best choice is to use a thin high atomic number target followed by a low atomic number electron stopper (BRAHME 1975) in order to spread out the high photon energy component as much as possible (cf Fig 10) The effect of this method is illustrated in Fig 12 in which a 1 5 mm tantalum target is followed by a 70 mm aluminium electron stopper High atomic number flattening filters are necessary to obtain broad beams at high energies since the thickness of the filter otherwise would be unacceptable The effect of a 40 mm lead filter used to produce a flattened beam of about 30 cm in diameter is illustrated in Fig 12 It is immediately seen that the beam penetration is decreased considerably by this filter even compared with the elementary beam The reason for this is the considerable loss of high energy photons and consequent contamination of the beam with low energy annihilation quanta and scattered photons [illegible] smaller field sizes intermediate [illegible] materials may also be used A 4 [illegible] was consequently used to produce [illegible] meter beam as also appears in Fig 1[illegible] [illegible] is now improved somewhat compared [illegible] elementary beam but the surface dose is increased However low and intermediate atomic number filters are excluded in broad clinical beams due to their excessive length and thus the advantages of broad high energy photon beams will be completely lost at these high energies

However broad photon beams could also be produced by scanning an elementary beam over a wide angular interval (cf Fig 2) The same mathematical technique as was used on the pencil electron beam (cf eq 2 and BRAHME & SVENSSON 1979) has been applied to the elementary photon beams The result from such a calculation for a composite target of 1 5 mm tantalum and 70 mm aluminium also appears in Fig 12 The considerably improved deep penetration compared with the filtered beams and the elementary beam is striking The small improvement in the build up region compared with the lead filtered beam and the degradation compared with the elementary beam should be expected It is due to the increased electron contamination in broad photon

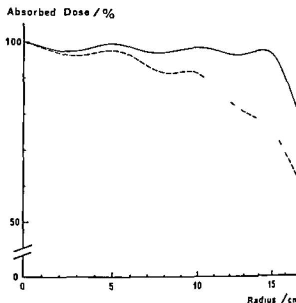


Fig 13 Lateral dose distribution obtained by 7×7 elemen[tary] photon beams of equal weight in a square grid (curve 1 [...] Curve 2 (—) obtained by doubling the weight at the edge

beams and it is automatically obtained in cor[rect] proportion by the integration procedure The rea[son] for the increase over the elementary beam curv[e is] that the contaminating electrons are spread ov[er a] larger area than the photons and thus their [...] effect is obtained in the integration procedure

When the scanned photon beam is used to p[ro]duce broad beams the grid or sieve technique m[ay] be of interest In order to investigate the dose d[is]tribution across the scanned beam a large numb[er] of crude beams were placed in a square grid patt[ern] and their lateral dose distributions were added T[he] result of this procedure is given in Fig 13 T[he] elementary beam used was that from a thick carb[on] target (cf Figs 10 11) Curve No 1 represents t[he] dose variation across the major axis in a square fi[eld] containing 7×7 grid points at a spacing of 5 cm [A] considerable dose fall off near the edges of the be[am] is obtained due to the decreasing number [of] neighbouring beams near the edge of the field Cur[ve] No 2 represents an almost perfect uniformity wh[ich] is obtained by doubling the weight of the elementa[ry] beams at the edge giving a field size of 30 cm×30 c[m] with a uniformity of ±1 5 per cent

From the high uniformity already at a grid spaci[ng] of 5 cm and the fairly narrow elementary beam fro[m] a thick carbon target it is evident that very th[in] targets have to be used to get a strong grid effect [...] photon beams This in turn would decrease the d[...] photon beams This in turn would decrease the d[ose] rate substantially thus making the scanned gr[id] technique less attractive for photon beams If [...]

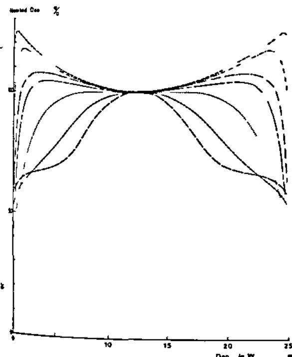


Fig 14 Central axis depth dose curves for parallel opposed broad photon and electron beams at a cross section of 25 cm Considerably decreased integral dose is obtained with electrons for a central target volume ^{60}Co (---) 4 (- -) 10 (- -) 25 (-----) 50 ( ) MV respectively 50 ( ) and 40 (----) MeV respectively

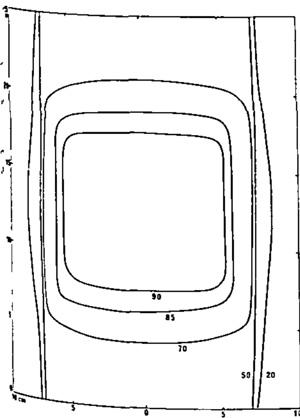


Fig 15

ever such narrow beams may still be of interest for use in the dynamic treatment of target volumes of complicated shapes without the need of moving heavy collimators

Parallel opposed beams

Given the capability of the racetrack microtron in delivering a wide range of radiation qualities in broad uniform beams of outstanding depth dose characteristics it is of interest to analyse how this may be used in treatment planning For the sake of simplicity and due to its importance for deep seated tumours the plane parallel opposed beam situation has been chosen

The high electron energies available make parallel opposed beams interesting for localized deep-seated tumours (OVADIA & UHLMANN 1960 ZATZ et coll 1961) In Fig 14 the central axis absorbed dose distributions for a 25 cm cross section are shown for a number of relevant beam qualities Of special interest is that the parallel opposed electron beams give a much smaller integral dose than photon beams in the same geometry provided the target volume is smaller than about 10 to 15 cm It is also possible to place the target volume eccentrically by using different electron energies or a combination of photons and electrons In Fig 15 the isodose curves for parallel opposed 50 MeV electron beams for the case of a central target volume are shown This distribution is based on the broad beam data in Fig 6 Due to the high electron energy used the penumbra at the target is still acceptable Furthermore it should be pointed out that the high energy considerably reduces the effect of disturbing inhomogeneities that normally appear at lower energies The isodose contours are thus not very different from the ideal dose distributions obtained with heavy charged particles like pions and protons with a dose to superficial tissues of only 60 to 70 per cent of the target dose

In addition to the advantageous distribution of absorbed dose an additional biologic effect may be obtained if the beam is produced by scanning a narrow electron beam of very high dose rate and short duration (~50 ns) This will give a low mean dose rate to the target volume where many individual beams overlap due to the pear shape of each individual narrow electron beam (cf Fig 3) The sur

Fig 15 Isodose contours (per cent) of 50 MeV parallel opposed electron beams Due to high electron energy the penumbra is fairly narrow and the influence of inhomogeneities quite small

face dose will on the other hand be given by a single high dose rate pulse which could give increased survival according to the high dose rate effect observed by TODD et coll (1968) A possible explanation for this effect could be the reduced importance of oxygenation at pulse lengths which are comparable with the life time of most radiation induced radicals (10 ns) A smaller influence of the presence of oxygen should then be expected since the relative concentration of competing radiation induced radicals is increased In addition this effect could also be used to explain the improved skin sparing observed by GRIEM et coll as their mechanically scanned narrow electron beam gave a much higher average dose rate to the superficial layers than to the tumour A further possible advantage with such a scanned electron beam is the already discussed grid effect

SUMMARY

The quality of broad high energy photon and electron beams can be improved considerably by the scanning of elementary narrow beams Dose distributions produced in this way have advantages with respect to improved dose gradients and therapeutic ranges for electrons and lower surface doses inc[illegible] depth doses and half value depths for photons Th[illegible]ility of giving the whole therapeutic dose in a sing[illegible] pulse of short duration is discussed as a new [illegible] to protect superficial tissues

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SHORT-TERM INTRA-ARTERIAL MITOMYCIN C IN HEPATIC METASTASES

W MATTSSON K JONSSON C HELLEKANT and L HALLSTEN

Untreated patients with hepatic metastases have a poor prognosis regardless of the site and nature of the primary tumour The median survival of patients with liver metastases from colo rectal carcinoma is 3 to 6 months (JAFFE et coll 1968 BENGMARK & HAFSTRÖM 1969 WOOD et coll 1976) and from mammary carcinoma the median survival is 2 to 3 months (DEVITT 1971 MATTSSON 1979) Furthermore if multiple organs are involved the length of survival depends on the extent of the hepatic metastases (JAFFE et coll)

By using systemic chemotherapy or radiation therapy of hepatic metastases no considerable increase of survival has been achieved although varying temporarily symptomatic improvement has been observed (SHERMAN et coll 1978)

Intra arterial chemotherapy of the liver is attractive since both experimentally induced liver tumours (BREEDIS & YOUNG 1954) and flow measurements in humans (GELIN et coll 1968) show that the malignancies receive almost all their blood supply from the hepatic arteries Local administration of cytostatic drugs intra arterially was introduced by KLOPP et coll in 1950 Thereafter intra arterial long term infusions have been used in patients with hepatic metastases (ARIEL & PACK 1965 ANSFIELD et coll 1975 OBERFIELD et coll 1979 PETREK & MINTON 1979) The results have been modest A median survival of the responders of about 6 to 10 months compared with 2 to 4 months in the non responders The rate of complications both to the technical procedure and to the treatment has been high Furthermore the complicated procedure can permit only a minority of the patients to receive this form of treatment because of lack of resources and the high costs However intra arterial combination chemotherapy (DOUGLAS 1979) combined intra arterial chemotherapy and irradiation (FRIEDMAN et coll 1979) and chemotherapy combined with starch microspheres (ARFORS et coll 1979 ARONSEN et coll 1979) have recently shown encouraging results

Since the early sixties angiography of various malignancies has been a routine procedure in this hospital in order to assess operability and prognosis Japanese experience with short term intra arterial chemotherapy has shown promising results (KINAMI et coll 1972 KINAMI & MIYAZAKI 1978) These facts initiated trials with a modified short term intra arterial Mitomycin C therapy in different tumours such as pulmonary carcinoma (HELLEKANT & SVANBERG 1978) gallbladder carcinoma (VON EYBEN et coll 1980) head and neck carcinoma (MATTSSON et coll 1977 ANDERSSON et coll 1979) pancreatic carcinoma (JONSSON et coll 1980) and other malignancies including hepatic metastases

Material and Methods

The material consisted of 30 patients admitted 1976 to 1979 to this Department of Oncology with

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progressive biopsy proven hepatic metastases of evaluable/measurable extension where conventional treatment failed or had not been given (Table 1) Six patients were non-evaluable because of early death not related to the treatment (3 pulmonary carcinoma 2 colon carcinoma 1 melanoma) Six were males (4 colon carcinoma 1 leiomyosarcoma 1 oesophageal adenocarcinoma) 18 were females (5 colon carcinoma 7 mammary carcinoma 3 ovarian carcinoma 1 carcinoid 1 malignant lymphoma 1 unknown primary tumour) The median age was 57 years (34–74) The median free interval was 0 (0–30 months) in colon carcinoma 41 months (20–70 [illegible]nths) in mammary carcinoma 32 months (22–180 [illegible]) in ovarian carcinoma and 0 months (0–184 [illegible]) in [illegible]arious malignancies respectively

Systemic chemotherapy of metastases had previously been [illegible] to 4/9 patients with colon carcinoma 6/7 w[illegible] [illegible]mmary carcinoma (all also endocrine res[illegible] [illegible]3 with ovarian carcinoma and 4/5 with [illegible] [illegible]nancies After angiographic demonst[illegible] tumour and its vascularity the in[illegible] [illegible]sions were given as selectively as [illegible] [illegible]mour feeding artery (arteries) i e [illegible] [illegible] or the coeliac trunk In patients in w[illegible] [illegible] lobe was supplied from an aberrant artery from the superior mesenteric artery or in whom the left lobe of the liver was supplied from the left gastric artery these vessels were selectively catheterized All catheterizations were performed from either femoral artery with percutaneous technique Angiography was carried out before each infusion After each infusion the catheter was withdrawn The patients were hospitalized for one day at the Department of Oncology during the treatment

In 3 patients only one infusion was given In 21 patients 2 to 9 infusions (median 3) were administered during a period of 2 weeks to 13 months At each course the patients were given 10 to 15 mg Mitomycin C dissolved in 100 to 150 ml of physiologic saline The infusion was given at a rate of 6 ml/min and the whole procedure was usually completed within 1½ hours

Before each course all patients were examined physically Karnofsky s index was assessed complete blood counts electrolytic status (potassium sodium creatinine urate in serum) liver enzymes (ASAT ALAT ALP GT) serum bilirubin were performed as well as radiography of the chest and angiography Before every second or third course liver scintigraphy was performed in patients receiving only one course within 4 weeks of treatment I[illegible] patients ultrasound examination of the liver was car[illegible]ried out before and after treatment

The results have been evaluated as follows Com[illegible]plete remission total normalization as demonstrat[illegible] at angiography liver scintigraphy ultrasound of th[illegible] liver laboratory examinations and physical cond[illegible] tion Objective response complete remission or a[illegible] objective improvement at angiography or scinti[illegible] graphy ultrasound of the liver and normalization o[illegible] liver enzymes (≥30% decrease of initial elevated values) and subjective improvement No indi[illegible] of progression as analysed with these methods wa[illegible] allowed No response unchanged condition or p[illegible] gression as recorded at angiography scintigraph[illegible]

Table 1

Short term intra arterial Mitomycin C treatment of hepa[illegible] metastases 1976 to 1979

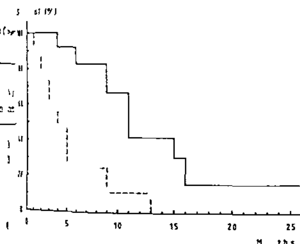
Primary tumour	No of patients	Evaluable patients	Total No of courses	Media[illegible]
Colon	11	9	36	3
Breast	7	7	[illegible]6	3
Ovary	3	3	6	[illegible]
Lung	3	0	–	
Various	6	5	[illegible]	4
Total	30	24	90	3

Table 2

Short term intra arterial Mitomycin C treatment of hepatic metastases from colon carcinoma OR=objective response NR=no response PD=progressive disease

Case	No of courses/ dose (mg)	Results	Survival (months)
1	7/95	OR	9
2	4/60	OR	6
3	5/65	OR	9
4	3/45	NR	13
5	7/100	NR	5
6	3/30	PD	10
7	3/45	PD	[illegible]
8	1/10	PD	4
9	1/15	PD	

Previously treated with systemic chemotherapy



Sh rt-term intra arterial Mitomycin C treatment in hepatic metastases Responders (—) Non responders (---) 0 001< p<0 01 (log rank test)

Table 3

Short term intra-arterial Mitomycin C treatment of hepatic metastases from mammary carcinoma CR=complete regression PR=partial regression NR=no response PD=progressive disease

Case	Previous chemo therapy	No of courses/ total dose (mg)	Results	Survival (months)
10	VACM	3/40	CR	15+
11	VAC 5-FU Mi	4/50	PR	11+
[illegible]	VACM	9/1?5	PR	15
14	VAC	4/40	PR	11
[illegible]	VACM	3/45	NR	5
[illegible]	CCNU L PAM	?/ 0	PD	2
16	VACM	1/10	PD	3

Table 4

Results of short term intra arterial Mitomycin C treatment of hepatic metastases

Primary tumour	No of objective regression/ No of patients
Colon	3/9
Breast	4/7
Ovary	?/3
Various	3/5
Total	12/24

ultrasound of the liver or increase of liver enzymes Survival was recorded from the start of the intra arterial chemotherapy

Results

An objective response was achieved in 12 of the 24 evaluable patients (Tables 2–4) The median survival was 11 months of the responders compared with 3 months for the non responders (0 001<p<0 01 log rank Figure) In 3 of the 9 patients with meastases from colon carcinoma an objective response occurred (Table 2) Among the non responders 2 patients had unchanged angiographic and scintigraphic appearance of the tumour as well as unchanged results of laboratory examinations at 4 and 7 months after beginning of treatment respectively Objective response was achieved in 4/7 patients with mammary carcinoma 2 patients are still alive in good condition without signs of hepatic metastases 11 and 15 months after beginning of the intra arterial treatment (Table 3)

Two of the 3 patients with ovarian carcinoma responded objectively and one of them is still alive at 28 months in a good state of health but with remaining tumour in the liver Each of the 3 patients with metastases from an unknown primary tumour leiomyosarcoma and adenocarcinoma of oesophagus respectively responded objectively No patient responded objectively after only one course of treatment while a symptomatic improvement was achieved in 9 patients Some regression of the tumour was registered in 8 of 12 responders after two courses although an objective response as defined did not occur until more courses were delivered (median 4 courses)

Side effects No complication of the technical procedure was encountered Subjectively the therapy was well tolerated Mild nausea easily treated with anti-emetics occurred in 10 patients during 36 courses (Table 5) Two patients had bronchopneumonia probably not treatment related (no leukopenia) The major problem was the cumulative thrombocytopenia which occurred at a median dose of 45 mg (10–85 mg) in 11 patients (Table 5) This thrombocytopenia was long standing and hampered further treatment in all these patients In 6 patients a normalization of platelets occurred within 3 months but in 5 the thrombocytopenia persisted for 3 to 11 months However no secondary bleedings occurred Otherwise no renal hepatic or other organ toxicity was observed

Complementary treatment after intra arterial Mitomycin C Two non responders of the 9 patients (Cases 4 and 5 Table 2) with hepatic metastases from colon carcinoma received 4 and 2 courses of combined intravenous 5 FU and Mitomycin C respectively also without objective regression The 4 responders among patients with mammary carcinoma who all had metastases also in other organs received treatment from 6 weeks after the third intra arterial treatment with Mitomycin C and when the objective regression of this therapy was already documented Two patients (Table 3 Case 11 with lytic bone metastases Case 13 with lytic bone metastases metastases of soft tissue and pleura) received weekly Adriamycin 20 mg The other 2 patients (Case 10 with pleural and bone metastases Case 12 with pleural pulmonary and bone metastases) were treated with VACM regimen and high dose medroxyprogesterone acetate respectively

Discussion

Short term intra arterial Mitomycin C chemotherapy of hepatic metastases is now a routine procedure with a negligible rate of technical complications The treatment is subjectively well tolerated Since an objective response was obtained in half of the patients with various malignancies it offers a measure of palliation which also has a positive cost benefit The majority of patients needed only one day of hospital care at each course The disadvantage of a high frequency of thrombocytopenia must however be considered The thrombocytopenia is not easily explained although it is a well known side effect of Mitomycin C (CROOKE & BRADNER 1976) It might be due to the high concentration of the drug administered

A low rate of platelet depression (not dose related) was observed in patients with mammary carcinoma treated with Mitomycin C administered intravenously (MATTSSON et coll 1980) In that regimen 10 mg Mitomycin C dissolved in 300 to 400 ml saline was infused over one hour every third week The critical median level at which thrombocytopenia occurred in the present material was 45 mg This is approximately the same as reported in a large survey from Japan (FRANK & OSTERBERG 1960)

The present results of short term intra arterial Mitomycin C treatment support the data from the original works in Japan (KINAMI et coll KINAMI & MIYAZAKI) and compares with the results obtained

Table 5

Side effects following short term intra-arterial Mitomycin C treatment of hepatic metastases (per cent in parentheses)

	No of patients	No of courses
Nausea	10/24 (42)	36/90 (40)
Thrombocytopenia	17/24 (71)	33/90 (37)
Infections	2/24 (8)	3/90 (3)

by long term infusions (ANSFIELD et coll OB FIELD et coll PETREK & MINTON) a techniq which has a considerably higher rate of seri complications (OBERFIELD et coll FORSBERG coll 1978) a high cost (PETREK & MINTON) and be offered only to a minority of patients becaus the complicity of the procedure

The median survival of patients with hep metastases from colo rectal carcinoma is shorte patients without free interval regardless of treate untreated (OBERFIELD et coll WOOD et coll) co pared with patients with a free interval In the pr ent material 5 of the 9 patients with metasta from colo rectal carcinoma had no free inter Three of them responded objectively and surv more than 6 months which is about double the pected survival without treatment However results of treatment in hepatic metastases fr colo rectal carcinoma is still unsatisfactory Beca of the encouraging results obtained by combi measures (irradiation and intra arterial che therapy FRIEDMAN et coll) and by enhanc the effect of cytostatic drugs via a decrease circulation (ARONSEN et coll) the intention is n to combine the short term intra arterial che therapy with starch microspheres and exter irradiation

The effect of intra arterial short term Mitomy C therapy in hepatic metastases from mammary ca cinoma and ovarian carcinoma resistant to system chemotherapy or endocrine treatment or both h been encouraging As the method is simple and w tolerated this treatment is now recommended as supplement to the intravenous chemotherapy

SUMMARY

Short term intra arterial Mitomycin C treatmen hepatic metastases from various malignancies was pe

formed in 34 evaluable patients An objective regression was obtained in 12 patients The median survival of responders was 11 months compared with 3 months in non responders No complication of the technical procedure occurred and the treatment was well tolerated The only side effect of clinical importance was thrombocytopenia which occurred at a median dose of 45 mg of Mitomycin C

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FROM THE DEPARTMENT OF GENERAL ONCOLOGY RADIUMHEMMET KAROLINSKA SJUKHUSET S 10401 STOCKHOLM AB KABI DIAGNOSTICA S 11287 STOCKHOLM AND THE DEPARTMENT OF HOSPITAL PHYSICS SÖDERSJUKHUSET S 100 64 STOCKHOLM SWEDEN

^{3} P PYROPHOSPHATE IN THE TREATMENT OF PERSISTENT METASTATIC BONE PAIN

B WERNER C ISACSON G LUNDELL C LONNBECK and B SODERBORG

Among the beta emitting radiopharmaceuticals used in the alleviation of pain caused by bone metastases probably the one in most common use is ^{3} P orthophosphate given either intravenously or orally (FRIEDELL & STORAASLI 1950 MAXFIELD JR et coll 1958 MILLER 1974) However the dose required for adequate palliation frequently causes such severe bone marrow depression as to prevent its adoption for routine use

As the beta energy and physical half life of ^{32}P are suitable other compounds of this nuclide were sought with a less marked tendency to cause bone marrow depression than the orthophosphate while being at least as effective as a palliative The pyrophosphate was considered to merit examination for this purpose Furthermore it was hoped that in the metabolism of the ^{3} P pyrophosphate the nuclide metabolites would prove to be incorporated in the neighbouring tumour cells with potential tumoricidal effect

Of intravenously administered ^{3} P pyrophosphate 40 per cent is known to be excreted by the kidneys In addition in preliminary in vivo experiments ^{32}P pyrophosphate uptake was at least 5 times greater in metastatic deposits in bone than in normal bone (SÖDERBORG & WERNER unpublished results) The radiation dose from 37 MBq (1 mCi) of ^{3} P pyrophosphate administered intravenously to adults was calculated to be of the order of 4 0 Gy in metastatic regions against 0 8 Gy in normal bone in which case the dose received by the bone marrow would be much lower

It was accordingly considered of interest to examine the haematologic and palliative effects of therapeutic amounts of ^{3} P pyrophosphate in patients with persistent metastatic bone pain (Permission was obtained from the Ethic Committee of Karolinska Sjukhuset and the informed consent of each patient was obtained)

Materials and Methods

^{3} P $Na_4P_2O_7$ was prepared by mixing solutions of H_3 ^{3} PO_4 (0 1 ml 37 GBq (1 Ci)/ml) and Na_2HPO_4 (120 mg 30 mg/ml) The mixture was evaporated to dryness and heated at 400°C for 2 hours The residue was dissolved in sterile water and then sterile filtered The solution was divided into single doses (370–555 MBq) which were freeze dried the product then being stable for up to 3 weeks as determined by paper chromatography For reconstitution sterile saline was used (0 9 mg/ml)

Eight patients (7 females 1 male) with persistent pain due to bone metastases from mammary carcinoma were given ^{32}P pyrophospate For at least 6 months before the administration none of the patients had advanced depression of the haematologic system or had received radiation therapy or

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Table
Lo est peripheral blood counts before and after administration of ^{32}P pyrophosphate Effect on pain and duration of relief

Case No	Hb (g×1)		Leukocytes (10⁹×1)		Thrombocytes (10⁹×1 ¹)		Effect	Duration (weeks)
	Before	After	Before	After	Before	After		
1	126	91	7 0	0 2	200	60	++	10
2	118	81	3 7	0 3	245	3 6	0	–
3	114	80	8 2	1 6	340	36	+	4
	126	92	7 1	4 4	149	70	+	6
4	133	130	6 1	7 0	255	250	+	5
5	106	80	4 7	1 8	196	16	++	10
	76	59	8 2	0 8	37	8	++	16
6	79	104	5 1	2 4	94	28	+	4
7	100	98	4 6	1 1	136	18	++	10
8	127	103	9 3	4 7	289	189	++	9

chemotherapy 444 MBq of ³ P pyrophosphate was given intravenously to the first 3 patients and 370 MBq to the other 5 once to 6 of the patients and on two occasions to the other 2

Peripheral platelet and leukocyte counts determination of the haemoglobin concentration and liver and renal function tests were performed before administration of the nuclide and afterwards at intervals of one week for the first 6 weeks and then in alternate weeks Bone radiography and bone scintigraphy using $^{99}Tc^{m}$ pyrophosphate were performed immediately before administration and again after 3 months

The effect of the therapy on pain was evaluated subjectively according to a three grade scale increased pain or no reduction (0) some alleviation (+) and complete alleviation with no further reliance on analgesic drugs (++)

Results

After the ^{32}P pyrophosphate therapy all but one of the patients reported some relief of pain (Table) It occurred first in the second week after the injection and lasted for 1 to 4 months The alleviation was greatest for patients with small metastases and less marked for those with collapsed vertebrae or extensive bone destruction

In all but one of the patients were the peripheral blood cell elements depressed For white cells and platelets this decrease began in the third week after the therapy the nadir occurred at 4 to 5 weeks and normalization was usually recorded at 6 weeks The erythrocyte counts were still slightly depressed afte 2 to 3 months

After the therapy the serum levels of alkalin phosphatase usually tended to be slightly reduce other biochemical tests of the hepatic renal an coagulation systems were unaffected In one case a elevated serum calcium level was normalized fo lowing treatment

In 6 patients no changes were found at bon scintigraphy or radiography up to 3 months afte treatment in the other 2 patients lytic areas wer calcified

In 2 patients deep venous thrombosis appeared i a leg 3 weeks after administration of the nuclide I both of them anticoagulant therapy was successful

Discussion

The various organic and inorganic ³²P com pounds that have been tested in therapeutic amo r in man have proved to be roughly equally effectiv in alleviating pain They have also produ ed sim lar side effects arising mainly from depressio of haematopoietic tissues (KAPLAN et coll 1960 POTSAID et coll 1978)

In the present series all but one of the 8 patie experienced less pain a proportion that is similar to those reported after use of other ³²P compou those reported after use of other ³²P compou with or without pretreatment with testosterone or other hormones (MAXFIELD JR et coll MILLER It is notable that the only patient not experien any pain reduction had essentially normal val es of serum alkaline phosphatases despite generalized

dissemination of the disease and an elevated uptake of ^{99m}Tc pyrophosphate in metastatic bone as demonstrated at scintigraphy

In all but one of the patients haematologic depression occurred serious in one case but not lethal The platelet count seemed to be most susceptible with a nadir at 3 to 4 weeks after the administration of ^{32}P At the same time also a profound but less marked depression of the leukocyte count occurred

In the comparison of various methods for relieving pain the selection of the patients is obviously important Patients receiving nuclides early in the course of the disease will probably experience a greater relief than those with extensive bone destruction Irrespective of any such selective factor in the present series the side effects were clearly too marked for ^{32}P pyrophosphate to be recommended for routine palliative treatment of persistent pain due to bone metastases From the observed side effects it would appear that the pyrophosphate molecule is broken down and that ^{32}P is then probably taken up by the bone marrow cells

SUMMARY

Eight patients with persistent pain due to disseminated bone metastases from mammary carcinoma were given about 370 MBq (10 mCi) of ^{32}P pyrophosphate on 10 occasions All but one of the patients experienced alleviation of pain lasting 1 to 4 months The side effects which derived mainly from haematopoetic tissue prevent the routine use of this compound

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SERUM CALCITONIN IN PATIENTS WITH OSTEOLYTIC AND OSTEOSCLEROTIC METASTASES FROM MAMMARY CARCINOMA

H E NIELSEN and C CH GADEBERG

Increased serum immunoreactive calcitonin (S iCT) concentrations have been found in patients with mammary carcinoma (COOMBES et coll 1976 MULHALD et coll 1977 OLSEN et coll 1978) COOMBES et coll found elevated S iCT in 23 of 28 patients with breast carcinoma and metastatic disease whereas only one of 13 with localized disease had increased levels and OLSEN et coll elevated S iCT in patients with osteolytic bone metastases as compared with patients with local tumour However 2 patients with osteosclerotic metastases had very low S iCT concentrations

In contrast to these authors ROUSSEAU et coll (1978) found marked hypercalcitonemia in a patient with osteosclerotic myeloma and suggested that osteosclerotic bone lesions could be induced by ectopic secretion of calcitonin by tumour cells Therefore it was considered of interest to investigate whether S iCT differed between patients with mammary carcinoma and osteolytic and osteosclerotic bone lesions and furthermore to compare biochemical and clinical values in these two patient groups

Material and Methods

The series consisted of 27 women with microscopically proven mammary carcinoma and with radiologically proven bone metastases 19 had osteolytic and 8 osteosclerotic metastases The mean age of the former group was 57 3 years (range 38–74) and of the latter 60 8 years (range 45–79) None of the patients received drug therapy or irradiation at the time of the present investigation All patients had normal renal function evaluated by serum creatinine

Serum concentrations of calcium phosphorus protein and calcitonin (S iCT) were measured after fasting overnight Serum calcium was corrected for individual variation in serum protein concentration (PEDERSEN 1973) and calculated as the calcium concentration corresponding to a protein level of 70 g/l (S calcium corr) S iCT was measured by a radioimmunoassay as described previously (NIELSEN et coll 1979 NIELSEN & OLSEN 1979) using a commercial antibody to synthetic human calcitonin (Calbiochem USA) and synthetic human monomer calcitonin (Ciba Switzerland) for standards and iodination The detection limit was 20 pg/ml Normal range was 0 to 120 pg/ml and detectable values could be measured in 85 per cent of normal males and 59 per cent of normal females The intraassay coefficient of variation was below 3 per cent at a serum concentration of 350 pg/ml and 14 per cent at a serum concentration of 30 pg/ml The interassay variation coefficient was 14 per cent at a S iCT concentration of 140 pg/ml

A group of 13 normal women with a mean age of 47 4 years (range 37–57) was used as control group for the S iCT values

Statistical evaluation Spearman s rank correla

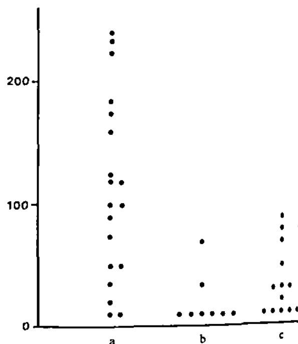
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tion test was used for correlation analysis The Mann Whitney U test was used for comparison of group means

Results

A significantly higher S iCT concentration was found in patients with osteolytic metastases as compared with patients with osteosclerotic (p<0 01 Figure) and with normal controls (p<0 01) No difference was found in S iCT between patients with osteosclerotic metastases and normals In the osteolytic group 11 of 19 patients appeared with S iCT values higher than the maximum value in the control group whereas in the osteosclerotic group all S iCT values were normal

One patient in the osteolytic group had hypercalcemia and 9 patients elevated S alkaline phosphatase None of the patients in the osteosclerotic group had hypercalcemia whereas 6 had increased S alkaline phosphatase No significant difference was found in the values of S-calcium S phosphorus or S alkaline phosphatase between the two groups (Table) nor any difference in the histologic classification of the tumour or in the localization of the bone metastases



Serum calcitonin in patients with mammary carcinoma a) Nineteen patients with osteolytic metastases b) 8 with osteosclero[tic] metastases c) Normal controls

Discussion

Increased S iCT was found in 11 of 19 patients with osteolytic bone metastases in contrast to normal S iCT concentration in all 8 patients with osteosclerotic metastases Previously a high frequency of elevated S iCT concentration was found in patients with metastatic disease from mammary carcinoma (COOMBES et coll 1976 OLSEN et coll)

RASMUSSON et coll (1978) compared S iCT in patients with osteolytic and osteosclerotic metastases Normal or undetectable S iCT was found [in] all but one of 26 patients and no difference w[as] demonstrated between patients with osteolytic a[nd] osteosclerotic metastases The finding of normal [or] undetectable S iCT values in most patients with o[s]teolytic bone metastases is in contrast to most oth[er] reports on patients with breast carcinoma (COOMB[ES] et coll 1976 MILHAUD et coll 1977 OLSEN [et] coll) It might be due to that the patients in th[e] series of these authors were treated with pre[...]

Table

Biochemical values (mean range) in patients with mammary carcinoma and osteolytic and osteosclerotic metastases

Metastases	S-calcium corr (mg/100 ml)	S phosphorus (mg/100 ml)	S alkaline phosphatase (units)	S-iCT (pg/ml)
Osteolytic (n=19)	9 74 (9 1–11 7)	3 6 ([illegible]6–4 7)	4[illegible]6 (101–3 [illegible]00)	11 (10- 40)
Osteosclerotic (n=8)	9 75 (9 1–10 6)	3 4 ([illegible]5–4 [illegible])	283 (149–590)	[illegible]1 (10–70)
P	NS	NS	NS	<0 01

were anti-oestrogen and cytostatics during the analysis period

Increased S iCT in patients with malignant disease other than medullary thyroid carcinoma has been explained by an ectopic CT production from the tumour (COOMBES et coll 1974 SILVA et coll 1974) or by a physiologically increased CT secretion from the thyroid (SILVA et coll 1975) to prevent osteolytic processes in the bones In patients with pulmonary carcinoma both mechanisms have been demonstrated (SILVA et coll 1974 1975) In vitro experiments with lung and breast carcinoma tissue (COOMBES et coll 1976) both types of tumour have been found to secrete CT The present finding of increased S iCT in patients with breast carcinoma with osteolytic but not in those with osteosclerotic metastases support the view that increased S iCT may be due to a physiologically increased CT secretion to prevent osteolysis rather than an ectopic production of CT

SUMMARY

Serum concentrations of calcitonin calcium phosphorus and alkaline phosphatase were measured in 19 patients with osteolytic metastases and in 8 patients with osteosclerotic metastases from mammary carcinoma A significantly higher mean S-iCT concentration was found in patients with osteolytic metastases as compared with those with osteosclerotic No significant difference was found in S-calcium S phosphorus or S alkaline phosphatase between the two groups

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PALLIATIVE IRRADIATION OF BRAIN METASTASES

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The treatment of brain metastases is discussed periodically as the search continues for the best means of palliation considering the patient s survival and quality of life hospitalization period treatment cost convenience to physician and patients etc As POSNER & SHAPIRO (1975) have stated it is a frustrating problem for the following reasons the tumors are difficult to control the evaluation of survival time is complicated by the fact that usually the metastases are widespread and many who respond to treatment of the metastatic brain tumor succumb to other complications of their systemic malignancy

The incidence of brain metastases varies from 2 2 to 35 per cent depending on the institution and the material examined Before CHAO et coll (1954) reported on the irradiation management of brain metastases palliation was achieved with surgery in selected cases (STÖRTEBECKER 1954) Since then radiation therapy has become the palliative treatment of choice as it achieves the same results as surgery without the high mortality rate observed with the latter The dose of radiation has varied from 15 Gy single dose (HINDO et coll 1970 JAZY & …ZON 1974 SHEHATA et coll 1974 YOUNG et coll 1974) to 55 Gy in 5½ weeks in a few cases (ORDER et coll 1968) although the commonly suggested dose is 30 Gy in 2 weeks (MONTAGUE 1973 POSNER 1974) The best palliative dose has remained unsettled Over the last 3 years in this institution 30 Gy in 2 weeks has been the most commonly used dose This appeared to provide adequate palliation in most cases however a recent wave of recurrences prompted us to review the results of this treatment schedule

Materials and Methods

The records of 121 patients with brain metastases evaluated for palliative irradiation from January 1976 to December 1978 were reviewed There were 70 males and 51 females ranging in age from 34 to 81 years with both the median and mean age at 59 89 were white 32 black

The lung was the most common site of the primary tumor (81/121 66 9%) Of these lung primaries were 36 (44 4%) well differentiated squamous cell carcinoma 15 (18 5%) poorly differentiated squamous cell carcinoma 21 (26%) adenocarcinoma and 9 (11 1%) oat cell carcinoma (Table 1) The breast was a distant second in frequency (14/121 11 6%) The primary tumor was unknown in 8 cases Fewer cases had other primary tumors (Table 1) The case with multiple myeloma was not a typical parenchymal lesion but was actually an extension of a large skull lesion into the brain causing the same signs and symptoms This lesion was debulked at craniotomy and the patient was referred for post operative irradiation

The presenting signs and symptoms appear in Table 2 As in several other series (BAKER 1942 LESSE & NETSKY 1954 MACGEE 1971 NISCE et coll 1971 STORTEBECKER) hemiparesis was the most common sign although this occurred in only 34 per cent Headache was the most common symptom

The diagnostic procedure used was scintigraphy with $^{99}Tc^{m}$ in 56 patients (46%) computed tomography in 48 (40%) and both procedures in 13 (11%) In 2 patients brain metastases were documented at angiography while in one case the EEG result was

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felt to be compatible with the diagnosis In one case the procedure was not specified

The distribution of patients according to the treatment modality used is given in Table 3 No irradiation was given to 8 patients 7 of these being in too bad condition due to their systemic disease to warrant even palliative irradiation In the eighth patient a craniotomy was performed with excision of a solitary lesion and no post operative radiation therapy was given Craniotomy was performed in 10 cases either to establish the histologic diagnosis or to rule out a primary brain tumor The operation was followed by irradiation Another patient had hydrocephalus secondary to an obstructive metastatic lesion in the fourth ventricle and a shunt was inserted before irradiation Irradiation alone was given to 101 p[illegible] 90 of these received 30 Gy in 2 weeks Se[illegible] p[illegible]tients who received 15 Gy or less actually [illegible] finish the radiation therapy due to progres[illegible]ir systemic disease

All [illegible]ted patients were treated with a 4 MV [illegible]ator except for one patient who was [illegible] Co All patients received whole brai[illegible] using lateral opposing fields The usu[illegible] was 3 Gy

[illegible] chemotherapy before and occasion[illegible] their irradiation course was administered t[illegible] patients This was not felt to have influ[illegible] results of irradiation and was not included in the analysis

Based on the initial history and physical examination the patients were classified as to neurologic functional status using the modification made by MONTANA et coll (1972) of the classification of ORDER et coll (Table 4) Evaluation of the functional status was made at the end of the treatment Patients were followed at 1 month and 3 months after treatment then at 6-month intervals thereafter

Results

The different diagnostic procedures revealed solitary lesions in 65 cases (53 7%) This was near the higher range of the 10 to 64 per cent incidence of solitary lesions reported by other authors (ASK UPMARK 1956 BAKER CHASON et coll 1963 LESSE & NETSKY RICHARDS & MCKISSOCK 1963) Thirty eight (58 5%) of these 65 solitary lesions were located in the frontal and temporal regions Both hemispheres were almost equally involved Multiple lesions were found in 54 patients (44 6%) In 2 cases brain scintigraphy was normal but cl[illegible] findings were highly suggestive of brain metasta[illegible] and the primary tumor was not controlled theref[illegible] they were treated palliatively In 53 of the 1[illegible]1 cas[illegible]

Table 1

Sites and distribution of primary tum[illegible]

Site		No	Per cent
Lung		81	66[illegible]
Squamous cell carcinoma well differentiated	36		
Squamous cell carcinoma poorly differentiated (including large cell)	15		
Adenocarcinoma	[illegible]1		
Oat cell	9		
Breast		14	11[illegible]
Unknown primary tumor		8	6[illegible]
Adenocarcinoma	4		
Undifferentiated carcinoma	[illegible]		
No histology	[illegible]		
Melanoma		6	5[illegible]
Colon		6	5[illegible]
Kidney		3	[illegible]
Urinary bladder		1	
Tongue		1	
Multiple myeloma		1	
Total		1[illegible]1	

Intracerebral extension of skull metastasis

Table 2

Presenting signs and symptoms

	No	Per cent
Hemiparesis	41	34
Headache	34	8
Aphasia	11	9
Visual impairment	10	8
Seizure	7	6
Ataxia	6	5
Confusion	6	5
Syncope	5	4
Vertigo	5	4
Personality change	4	3
Nausea and vomiting	3	[illegible]
Coma	[illegible]	
Hypopituitarism	1	
Memory loss	1	
Numbness of face	1	

Table 3

Distribution as to treatment

		No of cases
No treatment		7
Craniotomy only		1
Surgery + radiation therapy		12
Craniotomy + 30 Gy/2 weeks	10	
Craniotomy + 34 Gy/2½ weeks	1	
Shunt + 30 Gy/2 weeks	1	
Radiation therapy only		101
45 Gy/4 weeks	2	
40 Gy/3 weeks	1	
30 Gy/2 weeks	90	
0 Gy/1 week	1	
15 Gy/1 week or less	7	
Total		121

Table 4

Functional classification Adapted from ORDER et coll Radiology 91 (1968) 149 modification by MONTANA et coll Cancer 29 (1972) 1477 Reprinted with permission

Class	Definition
I	Neurologic findings minor or not present
II	Neurologic findings present but not a major factor no hospitalization required
III	Major neurologic findings present hospitalization required
IV	Profound neurologic deficit hospitalization essential

Table 5

Response to treatment based on neurologic function improvement

Neurologic function status				
Before treatment	After treatment			
	I	II	III	IV
I	31	[illegible]	1	
II	18	18	1	2
III	3	15	13	4
IV			1	4

reviewed brain metastases were diagnosed at the same time as the primary lesion 50 of these had pulmonary carcinoma

The distribution of patients as to their response to treatment based on improvement of the functional status appears in Table 5 Sixty per cent of the patients improved In certain Class II and III patients with several signs and symptoms amelioration of some symptoms occurred but little or no improvement of their other neurologic deficits Those patients were kept in their pretreatment classification A few of these patients improved further during the course of their follow up Interestingly the tabulation of the functional status shows a greater number of the present patients in Class I compared with those of MONTANA et coll ORDER et coll and SHEHATA et coll Most of the patients in this class had symptoms of headache aphasia visual impairment and other minor symptoms which were temporarily relieved by steroids Therefore their neurologic examination was unremarkable at the time of evaluation for radiation therapy

Only seven of the irradiated patients failed to finish their treatments One of these patients belonged to Class II 3 to Class III and 3 to Class IV before treatment Six of these 7 patients had progressive systemic disease at the time of whole brain irradiation which caused their rapid deterioration All of these 6 cases had primary pulmonary carcinoma

The overall recurrence rate was 17 7 per cent (Table 6) with pulmonary lesions comprising more than half of these recurrences The overall rate of retreatment was 8 8 per cent (10/113) Considering the recurrences in relation to their representative number in the patient population only 14 8 per cent of the metastases from the pulmonary tumor recurred compared with 42 8 per cent of those from the breast tumor Only half of the recurrences from these respective sites were retreated In the other half the metastases were noted in the terminal stages of the disease The average time between the initial brain irradiation and retreatment was 10 8 months for the metastases from lung tumors and 5 3 months for those from breast tumors The range for the whole group was 2 5 to 15 months Of these recurrences 7 developed within 3 months from first treatment Four of these 7 cases lived for a few more weeks while the other 3 died of their primary disease around the same time that their brain recurrence was noted The reason for the longer interval noted in

Table 6

Recurrences and retreatment

Primary tumor	Recurrences		No re treated	Average time between treat ments (months)	Improve ment rate
	No	Per cent			
Lung	12/81	14 8	6	10 8	5/6
Breast	6/14	42 8	3	5 3	3/3
Melanoma	1/6	16 6	–	–	–
Unknown	1/8	12 5	1	–	1/1
Total	20/113	17 7	10		9/10

Table 7

Comparison of the present results with other series

Series	No of cases	Dose (Gy)	Improve ment rate (per cent)	Median (months)	Mean (months)	One year (per cent)	Per cent retreated
CHAO et coll (1954)	38	30–40/3–5 weeks	63	–	8 2+ 4 6++	–	10 5
ORDER et coll (1968)	108	25–55/2½–5½ weeks	60	6	6 3	16	14 8
NISCE et coll (1971)	376	35/3–4 weeks	80	~6	–	~16	12 5
JAZY & ARON (1974)	18	10 single dose	55	–	3 2	0	–
MONTANA et coll (197)	47	30/2 weeks to 45/4½ weeks	56	~4	–	~12	–
SHEHATA et coll (1974)	81	10 single dose*	69	–	5 0	~? 5	47
HORTON et coll (1971)	44	40/3–4 weeks	61	3 5	–	–	–
POSNER (1974)	44	–	–	4	3 5	9	–
BORGELT et coll (1980)	910 (1 st)	30–40/2–4 weeks	47	4 5	–	–	–
	902 (2nd)	20–40/1–3 weeks	52	3 8	–	–	–
Present series	113	30/12 weeks	60	3	5 3	13	8 8

75 per cent of cases
67 per cent of cases
80 per cent of cases
+ responders survival from onset of intracranial symptoms
non-responders survival from onset of intracranial symptoms

the cases with metastases from lung tumors is not known although in 5 of the retreated cases the primary tumor was controlled The initial response to retreatment was good in 9 of the 10 patients with recurrence although the duration of remission after retreatment was quite variable Four cases with metastases from lung tumors and one patient with metastasis from a breast tumor are still alive 2 to 3½ months after retreatment The other patients lived from 1 to 9 months after retreatment

The overall survival rate in this series determined from start of treatment was 50 per cent at 3 months and 13 per cent at one year (Table 5) The 6-m[...] survival was 29 per cent The inclusion of the [...] tients who did not complete their treatments a[...] those who received 40 Gy did not influence t[...] median or one year survival The survival was t[...] same with the 53 cases whose primary lesions we[...] diagnosed at the same time as the brain metastas[...]

The effect of surgery on survival of those patien[...] who were given combined treatment was variab[...] The representative numbers were too small to wa[...] rant a meaningful analysis However 2 patients wi[...] metastases from lung tumors and good neurolo[...]

ction even the combined treatment are alive months and 2 years after treatment

The incidence of acute complications was insig icant Three patients had initial nausea and vomit ? had increased headache and one had lethargy pite the use of decadron These symptoms were eved by increasing the steroid dose One patient eloped intense scalp erythema which was con ered to be potentiated by concomitant chemo rapy using adriamycin

Discussion

The review revealed the same percentage of sig icant short term palliation as other authors have ind (Table 7) It appears therefore that 30 Gy in ks is an adequate dose for this purpose The ignment of 30 per cent of the present patients to ss I before treatment did not affect the palliative ult since all but 3 of them remained in that func nal level after treatment with significant amelio ion of their symptoms This observation was con ered an improvement as suggested by ORDER et l The median survival of 3 months was shorter n in other series (BORGELT et coll 1980 HORTON coll 1971 MONTANA et coll NEWMAN & HAN s 1974 NISCE et coll ORDER et coll POSNER) this may be due to the large number of cases h metastases from lung tumors whose median vival reflected that of the whole patient popula n The 1 year survival of the present patients was ilar to the approximately 12 per cent in the series MONTANA et coll

The us of shorter courses of 10 Gy single dose 5 Gy in 2 fractions as the initial palliative treat nt has been reported by some authors (HINDO et l JAZY & ARON SHEHATA et coll YOUNG et) showing the rate of initial neurologic im vement to be comparable to that obtained with lower daily dose fractionated schedules (Table However these shorter courses were associated h more severe acute complications and shorter vival (HINDO et coll JAZY & ARON YOUNG et l) Nevertheless there may still be place for this imen in certain cases with poor overall prognosis l rapid deterioration YOUNG et coll advise inst such treatment courses in patients with rk dly increased intracranial pressure

The results of two randomized investigations nparing 5 treatment schedules from 20 Gy in 2 eks to 40 Gy in 4 weeks have recently been re ported by BORGELT et coll Comparable results were obtained in all the treatment schedules using differ ent parameters The rate of neurologic improvement was 47 per cent in the first series and 52 per cent in the second A more rapid improvement was ob served in patients who received the shortest treat ment schedules (30 Gy/2 weeks in the first series and 20 Gy/1 week in the second series) Patients with a controlled primary tumor and with the brain as the only site of metastasis had a more favorable prog nosis It was suggested that these patients may be nefit from higher doses The present review re vealed that some of the patients in the same category had recurrence of the brain metastases even up to 15 months after the initial brain irradiation ORDER et coll also suggested that a higher initial dose may be necessary in selected cases to prevent retreatment and prolong the duration of improvement

The optimum higher dose still remains to be de termined ORDER et coll suggested an additional 10 to 15 Gy with reduced ports in patients given 25 to 40 Gy to the whole brain initially MONTANA (1979) gave 30 Gy in 2 weeks and added 6 Gy with a reduced port in cases with solitary lesion A current series comparing 50 Gy in 4 weeks and 30 Gy in 2 weeks for the patients with favorable prognosis has been published by BORGELT et coll

In those series where re irradiation was men tioned this was done in up to 47 per cent of their cases (Table 7) The number of retreated cases in those series indicated that not all of the patients with recurrences were re irradiated The retreatment dose was usually not discussed in the other series al though in those who used the single dose of 10 Gy it would be assumed that they used the same dose In the present series only half of the patients with recurrences were treated from 15 Gy in a week and a half to 30 Gy in 3 weeks were given with good results particularly in those patients who recieved a retreatment dose of at least 25 Gy The size of the retreatment fields depended on the extent of the recurrent lesion In most cases the whole brain had to be re irradiated

The effect of surgery on the results of treatment was not clearly evident as the number of patients in this group was relatively small The same observa tion was made by ORDER et coll in their series MONTANA et coll did not find any significant differ ence in survival between surgery plus irradiation and irradiation alone POSNER showed that patients treated by surgery and irradiation had a longer sur

vival than those given either rapid or slow irradiation alone Despite the variable results surgery may continue to be indicated in cases where the nature of the lesion is uncertain and biopsy confirmation is necessary (POSNER WILSON 1977) The improvement in the accuracy of scintigraphy and computed tomography in distinguishing metastatic disease from other intracranial lesions may further diminish the role of surgery in this aspect

In summary it appears that 30 Gy in 2 weeks is an effective palliative dose in most cases of brain metastases However in selected cases with good performance status primary tumor controlled and the brain is the only site of metastasis higher doses should be considered Shorter high dose courses also may be used depending on the severity of the patient s general condition The indications for surgery have t be considered on an individual basis

SUMMARY

Th l patients with brain metastases from a 3 ye reviewed Radiation therapy was given to per cent received 30 Gy in 2 weeks Be 31 were operated upon Significant neu ment was obtained in 60 per cent of the irr recurrences retreatment and the neces n l doses in selected cases are discussed

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TRANSIENT INTESTINAL ISCHAEMIA INDUCED BY DEGRADABLE STARCH MICROSPHERES

Experiments in the cat

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The basic concept of radiation therapy is tumour eradication without unacceptable injury to normal tissue. This is possible when the tumour is more sensitive to radiation than the normal tissues, i.e. when a therapeutic ratio exceeding one exists. Such a ratio can be raised by increasing tumour sensitivity or by decreasing normal tissue sensitivity.

The search for tumour specific sensitizers has not up to now resulted in clinically applicable methods. As oxygen plays an active role in the formation of free cytotoxic radicals mediating the biologic effect of commonly available ionizing radiation for clinical use (photons and electrons), the oxygen tension in the tissues can modify the radiation effect (GRAY et coll. 1953). The sensitivity of hypoxic cells in tumours can theoretically be raised by hyperbaric oxygen treatment at the time of irradiation, and the radiation sensitivity of the normal tissues can be decreased by methods causing hypoxia.

The hyperbaric oxygen treatment principle has been clinically tried (CHURCHILL-DAVIDSON et coll. 1957) but the results have not been convincing, and on account of complications this method is no longer used. Other methods increasing the oxygen content in the tissues by means of peroxide infusion have also been abandoned.

Protection of normal tissues by induction of hypoxia has recently been described. PENN et coll. (1975) demonstrated effective hypoxic protection to radiation of the gut induced by clamping the mesenteric arteries in dogs. STECKEL et coll. (1969 a, b) and JOHNSON et coll. (1968) demonstrated a similar protection of the gut and kidneys using vasoconstricting agents.

ARFORS et coll. (1976) in pigs induced a transient hypoxia in the small intestine by the injection of degradable starch microsphere (DSM) into the superior mesenteric artery. FORSBERG (1978 a) demonstrated a radiation protective effect on the hind foot and the same author (1978 b) as well as FORSBERG & JUNG (1978) found a similar effect in the small intestine of the rat after hypoxia induced by degradable starch microspheres. FORSBERG et coll. (1979) also demonstrated this protective effect against the development of late fibrosis in the gut wall. These experiments clearly show the possibilities of the hypoxic method for radiation protection already discussed by FORSBERG (1978 a).

In clinical radiation therapy the intestine and kidney are examples of comparatively highly sensitive tissues. Radiation injury to these organs with subsequent intestinal ulcerations, necrosis and stenosis or renal functional disturbances and hypertension may make it impossible to deliver a sufficient dose to intra-abdominal or retroperitoneal tumours.

The possible clinical use of hypoxia induced by injected degradable starch microspheres for protection of normal tissues unavoidably exposed to ionizing radiation awaits simple, reliable methods to monitor the induced ischaemia. It is not possible to

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use blood flowmeters or oxygen tension meters or directly observe the organ colour at repeated treatment sessions in patients

The present investigation was intended to assess in the cat (1) the value of a scintigraphic procedure to monitor the degree and duration of intestinal blood flow obstruction induced by the injection of $^{99}Tc^{m}$ labelled degradable starch microspheres into the superior mesenteric artery (2) the intestinal ischaemia induced by the starch sphere injection and (3) the intestinal and hepatic degradation of the starch microspheres

Materials and Methods

Animal preparation Seven adult male or female cats weighing 3 0 to 5 1 kg were used The animals were fed on water only during 12 hours before an experiment The cats were anaesthetized with sodium pentobarbital (40 mg/kg) and kept under light endotracheal anaesthesia after tracheostomy A cannula (OD 1 02 mm) was inserted into the inferior vena cava through a femoral vein A second cannula was placed in the left ventricle of the heart through the right carotid artery and a third was inserted into the abdominal aorta through the left femoral artery and secured with its tip just above the bifurcation A midline laparotomy was performed and the main trunk of the superior mesenteric artery was isolated and prepared for measurement of the flow After isolation and ligation of the distal end of the small ileocaecal branch of the superior mesenteric artery a plastic cannula (OD 0 63 mm) was inserted and advanced 3 to 5 mm in the proximal direction and fixed in this position with the cannula tip pointing into the main trunk of the superior mesenteric artery for injection of degradable starch microspheres Care was taken to secure that the tip of the cannula and the electromagnetic flow probe in the proximal part of the superior mesenteric artery were separated by at least 2 cm The arterial blood pressure and heart rate were recorded by means of a Statham pressure transducer P23De connected to a Hewlett Packard 7758 A Recorder The animals were placed on a heating pad and the rectal temperature maintained at 37 to 38°C

Degradable starch microspheres (DSM) with diameters of 37 6±5 9 μm suspended in 0 9% saline to a concentration of 13 2×10⁶ spheres/ml (generously supplied by Pharmacia AB Uppsala Sweden) were used to produce mesenteric arteriolar occlusion Before each experiment about 5×10⁵ sphe[res] were labelled with $^{99}Tc^{m}O_4$ (DJURSÄTER 1979) t[o] specific activity ranging from 1 5 to 16 3×10 Bq a[nd] mixed with unlabelled spheres in a Whirlmix[er] When the microspheres were sedimented by ce[n]trifugation less than 10 per cent of $^{99}Tc^{m}$ label r[e]mained in the supernatant The labelled DSM we[re] then resuspended in saline before use Each anim[al] recieved a dose of 27×10⁶ DSM in a volume of [ ] ml The injection time ranged from 75 to 105 se[c]onds

Carbonized microspheres (CMS 3 M Compa[ny] St Paul Minnesota USA) 14 8±1 3 μm diameter and labelled with ^{46}Sc ^{51}Cr ^{85}Sr or ^{141}Ce were used to measure blood flow per g of tissue Th[e] microspheres were suspended in 10% dextran wi[th] addition of Tween 80 to prevent aggregation Th[e] suspension was sonificated and mixed thoroughly [in] a Whirlmixer immediately before the injection Th[e] suspensions contained approximately 1 2×10⁶ CM[S] per ml and were injected into the left ventricle of th[e] heart in a dose of 0 2 ml/kg body weight

Tissue blood flow was determined by intracardi[ac] injection of CMS and simultaneous withdrawal of [a] reference blood sample from the aorta at a consta[nt] rate as described by HEYMANN et coll (1977) Th[e] net activity of the blood and tissue samples we[re] determined and the blood flow was calculated fro[m] the following formula

$$QT=QR\times\frac{\text{cpm T}}{\text{cpm R}}$$

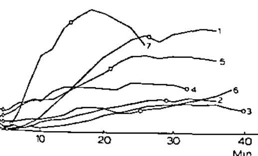
where QT=blood flow in tissue sample QR=bloo[d] flow in aortic reference sample cpm T=counts/min/g tissue cpm R=counts/min in reference bloo[d] sample

Cardiac output was also determined by the mi[cro]sphere technique according to the following for[]mula

$$CO=QR\times\frac{\text{cpm I}}{\text{cpm R}}$$

where QR=blood flow in aortic cannula duri[ng] withdrawal (1 03 ml/min) cpm I=counts/min o[f] total amount of injected microspheres and cpm R=counts/min in reference blood sample Tissue [and] blood samples were analysed in a multicha[nnel] gamma detector (ICN Instruments SC 7?2 Oakland California USA)

Intestinal tissue samples were taken at 9 levels



Electromagnetically measured blood flow (ml/min) in the r mesenteric artery before and following injection of ^{99}T labelled DSM into the artery The symbol O de arbonized microsphere tissue flow determinations

Table 1

magnetically measured blood flow (ml/min) in the supe rior mesenteric artery following DSM injection

	DSM injection		
	Before	3 min after	26 min after
	105	2	140
	65	0	47 5
	110	3 5	38
	58	22 5	70
	82	28	112
	45	12	34
	87	5	125
SEM	79±9	10±4	81±17

0 to 90 cm from the ligamentum of Treitz In sample the mucosa was separated from the ularis by simple stripping and the two layers analysed separately Microscopic examination d that the mucosa samples were composed of llowing layers mucosa muscularis mucosae ubmucosa An occasional sample contained strips of smooth musculature The muscularis es contained only smooth musculature and the l lining

m amylase was determined by standard ds (ZINTERHOFER et coll 1973) and expressed itrary units (normal range in man 40–140

imaging Activity emitting from the ab al area was recorded by a Pho/Gamma camera ear Chicago Illinois USA) connected to a Je Nukab computer (Digital Equipment Cor on Maynard Massachusetts USA) The in l and hepatic areas were identified by means of a radioactive marker By computer integration the accumulated number of counts over each area was determined at 3 min intervals for at least 27 min following the injection of ^{99}Tc^{m} labelled DSM into the superior mesenteric artery During prelimi nary experiments it was confirmed that the CMS used for tissue flow determinations contributed by less than one per cent to total abdominal activity recorded by the gamma camera The ^{99}Tc^{m} isotope in blood and tissue samples was permitted to decay for at least 7 days or 28 half lives and the samples were then counted for CMS activity

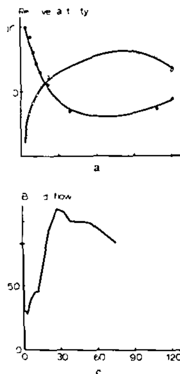
Arterial blood flow in the superior mesenteric ar tery was recorded by a flow probe with a diameter of 2 6 mm positioned over the artery and connected to a square wave electromagnetic flowmeter (Carolina Medica Electronics 501 King North Carolina USA)

Experimental procedure After instrumentation each animal was placed with the center of the abdo men upwards facing the center of the counting head of the gamma camera The animal was not moved during the subsequent experiment The first CMS injection was performed The electromagnetic probe on the superior mesenteric artery recorded blood flow continuously At time zero the ^{99}Tc^{m} labelled DSM were injected into the superior mesenteric ar tery through the cannula placed in its ileocaecal branch The flow was recorded at one min intervals From 2 to 4 5 min following the injection of DSM a second CMS tissue flow determination was carried out in each animal After 14 5 to 40 min when the mesenteric flow was restored to a stable plateau as evaluated from the individual electromagnetic flow recordings over the superior mesenteric artery a third tissue flow determination was performed The animal was then killed by intracardial injection of potassium chloride The liver pancreas spleen and both kidneys were removed and immediately counted for total activity by the gamma camera

Statistics Each cat served as its own control Student s two tailed t test for paired data was used to calculate statistical probability A common test for linear regression was also used for evaluation of the results A p value less than 0 05 was considered *statistically significant*

Results

Changes in arterial flow The changes in arterial blood flow in the superior mesenteric artery in the



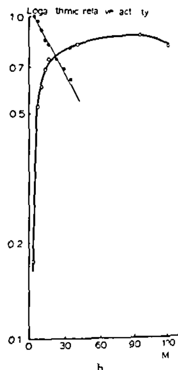


Fig 2 Observations obtained in cat No 5 a) Relative activity (in per cent) recorded over intestinal and hepatic areas 3 to 120 min following injection of Tc^m labelled DSM into the superior mesenteric artery Activity count over the intestinal area 0 to 3 min after DSM injection=100% by definition b) Se[mi]logarithmic plot of the data presented in Fig 4 (100% [...] c) Electromagnetically recorded blood flow (ml/min) in [the] superior mesenteric artery

seven experimental animals appear in Fig 1 Each animal was observed until the mesenteric blood flow reached pre injection control levels or was stabilized The mesenteric flow decreased to a minimum [...] 1 1 to 4 min following DSM injection and gr[adually] [was] recovered thereafter Control flow value was completely recovered 26 min after the DSM injection in 6 cats with 3 animals having reactive hyperaemia In one cat (No 3 Table 1) complete recovery did not occur although flow increased markedly from the minimum level One animal (No 5) was observed for two hours and had a moderate reactive hyperaemia followed by a gradual decline towards control flow level which was reached after 75 min (Fig 2c) Although the variation between the individual cats was considerable immediate and marked initial flow reduction after the DSM injection followed by flow restoration was consistently reproducible

Changes in tissue flow Intestinal CMS tissue flow was repeatedly determined at 9 consecutive levels in samples taken separately from the mucosa and the muscularis (Fig 3) Immediately before DSM injection a control CMS tissue flow determination was obtained 2 to 4 5 min after the DSM administration the minimum flow level was determined A recovery CMS tissue flow determination was performed when the electromagnetic blood flow had reached eith[er] pre DSM injection levels or a stable plateau phas[e] Due to individual variations in restoration of mese[n]teric arterial flow the time for the third determin[a]tion ranged from 14 5 to 40 min after the DSM inje[c]tion (Fig 1) The determination of the tissue fl[ow] demonstrated a highly significant reduction of th[e] mucosal flow immediately following injection [of] DSM In 2 of the cats the reduction in tissue flow [in] the distal ileum was much less than that observed [in] the proximal region

A trend towards slightly reduced mucosal reco[v]ery flow in the jejunum and part of the ileum w[as] evident when compared with pre DSM injectio[n] control values However at no intestinal level d[id] the difference reach statistical significance (p>0 05 In contrast tissue flow determinations in the m[us]cularis regularly showed reactive hyperaemia a[l]though the difference between control and recover[y] flow values only reached statistical significance [at] levels 10 60 and 70 cm aboral to the ligamentum [of] Treitz (Fig 3)

CMS tissue flow values in the liver pancreas [and] spleen were not significantly altered by the DS[M] injection and cardiac output remained un[changed] (Table 2) However renal tissue flow was sign[ifi]cantly reduced Thus although intra arterial inje[ction]

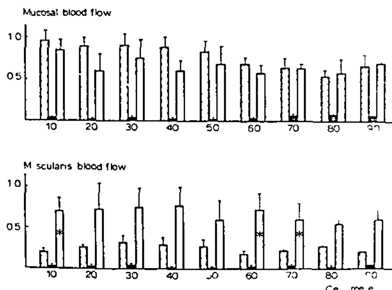


Fig 3 Tissue flow (carbonized microsphere distribution) in small ... nal mucosa (upper graph) and muscularis (lower graph) be... (□) ? to 4 5 (■) and 14.5 to 40 min (□) following ...ection of DSM into the superior mesenteric artery Mean and SEM of 7 observations obtained at different distances aborally from the ligamentum of Treitz (in cm) are given =statisti ally significant difference (p<0 0) between control and recovery values

...ion of DSM profoundly altered intestinal blood f... the systemic circulation was not compromised ...ept for a transient effect on the renal blood flow

The CMS tissue flow method and the electro ...netic method measure blood flow at separate le...els in the arterial circulation For each experi ...tal animal three pairs of simultaneous CMS tis ...ue flow determinations and arterial probe flow ...adings were obtained demonstrating a positive correlation between the two methods (Fig 4)

Degradation and distribution of starch micro-spheres Emission from the hepatic and intestinal ...s following $^{99}Tc^m$ labelled DSM injection was ...ontinuously recorded Since the tracer must pass ... mesen...eric vascular bed to reach the liver intes ...l activity during the time interval 0 to 3 min ...llowing DSM injection was defined as 100 per cent ...d accordingly the relative activity remaining in the intestinal area and the activity accumulating in the liver was calculated It is recognized however that a minute fraction of $^{99}Tc^m$ indicator escaped from the intestinal to the hepatic area during the first 3 min interval making the defined 100 per cent slightly less than the amount of tracer actually injected into the superior mesenteric artery

During the first 27 min following $^{99}Tc^m$ labelled DSM injection intestinal relative activity decreased while hepatic activity increased (Fig 2a) in all animals The individual semilogarithmic plots of intestinal relative activity transfer demonstrated initial exponential removal of $^{99}Tc^m$ and hence of DSM (Figs 2b 5) while the liver showed rapid initial uptake of $^{99}Tc^m$ with a prolonged excretory phase (Fig 2b) At the end of the experiment the liver had accumulated approximately 80 per cent of the total activity present in the liver spleen pancreas and

Table 2

T ssue flow (ml/min/g) and ca diac outpt t (ml/min) (carbon ed microsphere distribut on) determined befo e (control) 2 to 4.5 min and 14 5 to 40 min after injection of $^{99}Tc^m$ labelled DSM into the superio mesenteric arter Mean ± SEM of 7 animals Asterisk denotes a significant difference (p<0 05) from control values

DSM injection	Liver	Kidneys	Spleen	Pancreas	Cardiac output
Before	1 9±0 9	1 99±0 ?8	? 67±0 5	0 65±0 11	446±61
to 4 5 min after	1 1?_0 48	1 0±0 1	? 66±0 48	0 56±0 15	478±55
14 5 to 40 min after	1 6~0 6	? 14±0 18	2 33±0 39	0 99±0 43	408±60

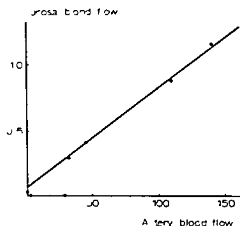


Fig 4 Relationship between small intestine mucosal blood flow (ml min g) CMD and electromagnetically measured blood flow (ml min) in the superior mesenteric artery Determinations performed times in each of 7 animals Mucosal flow values represent mean of observations made at 9 levels of the small intestine

Table 3

Relative $^{99}Tc^m$ activity (per cent) in isolated abdominal organs counted by gamma camera after killing the animal The sum of activity in the liver pancreas spleen and both kidneys defined as 100 per cent Mean ± SEM for 6 animals

	Relative activity
Liver	80±5
Pancreas	7±1
Spleen	?±0
Kidneys	11±4

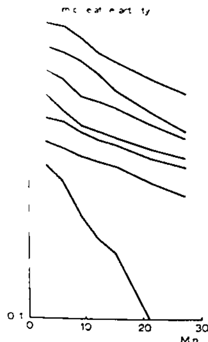


Fig 5 Semilogarithmic plots Decline of relative activity over the intestinal area following injection of $^{99}Tc^m$ labelled DSM into the superior mesenteric artery Log activity counted over the intestinal area for 0 to 3 min after DSM injection=1 0 The starting points for each curve on the ordinate scale are separated by a fixed interval for separation of the curves Values on the ordinate scale are valid for cat No 1

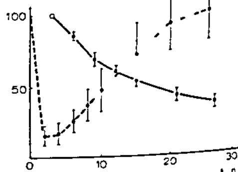


Fig 6 Relative activity (per cent) recorded over intestinal area (— —) and relative blood flow (per cent) in the superior mesenteric artery (-- --) following injection of $^{99}Tc^m$ labelled DSM into the artery Activity counted over intestinal area 0 to 3 min following and arterial flow preceding DSM injection is defined 100% respectively Mean and SEM of 7 animals

kidneys (Table 3) The gamma camera images taken 3 18 and 111 min following the injection of $^{99}Tc^m$ labelled DSM showed no abdominal hot spot outside the intestinal or hepatic areas (Fig 7) One cat was observed for 120 min following the DSM injection and showed a late increase in intestinal activity with a concomitant decrease in hepatic activity indicating recirculation of tracer (Fig 2a b)

As relative activity recorded over the intestinal area decreased relative arterial flow (per cent of pre DSM injection control value) in the superior mesenteric artery increased after a plateau of minimum flow lasting for 4 to 6 min (Fig 6)

Serum amylase activity (mean 1450 range 10.. 2100 arbitrary units) was not influenced by anim.. preparation or DSM administration Thus a ...

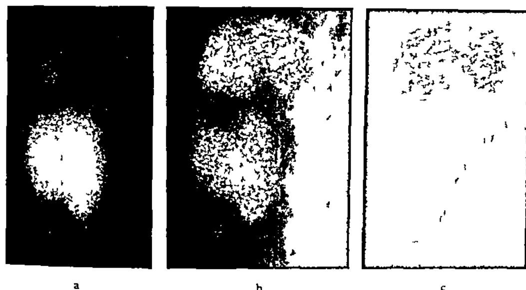


Fig 7 Gamma camera images 3 18 and 111 min following injection of ^{99}Tc labelled DSM into the superior mesenteric artery demonstrating tracer transfer from the intestinal to the hepatic area (cat No 5)

thetized cats possess 7 to 15 times higher amylase activity than do resting humans when the amylase activity is determined by the nephelometric method of ZINTERHOFER et coll

Discussion

Temporary block of mesenteric blood flow in animals or man may be achieved by laparotomy and direct clamping of the superior mesenteric artery or by catheterization procedures using intra arterial inflatable balloons The extensive collateral circulation made possible by the arcading mesenteric arteries will be expected to shorten the length of small intestine rendered ischaemic by these methods A theoretical advantage of degradable starch microspheres is that they will be carried by the blood stream through the arteries and finally embolize medium size arterioles (ARFORS et coll FORSBERG 1978b) thus minimizing blood flow through collateral vessels

In the present experiments selective DSM injection into the superior mesenteric artery immediately and markedly reduced blood flow in the main arterial trunk and in the intestinal mucosa and muscularis in all but 2 animals blood flow was blocked along the entire small intestine from the duodenum to the caecum When blood flow in the superior mesenteric artery is arrested mucosal oxygen is depleted in about 4 min in pigs (ARFORS et coll) and extensive mucosal injury develops in 1 to 2 hours in animal models (ROBINSON & MIRKOVITCH 1972 RIJKE et coll 1976) progressing to mesenterial infarction if ischaemia persists However mesenteric arterial flow and blood flow in the intestinal mucosa and muscularis were restored at all levels of the small intestine after 26 min in the present experiments Although one cat (No 3) showed a 70 per cent reduction in arterial flow absolute recovery flow was sufficiently high to make intestinal ischaemic damage unlikely

Intestinal muscle spasm induced by ischaemia (MARSTON 1963 ARFORS et coll) would increase post ischaemic intestinal oxygen demand and could explain the reactive hyperaemia consistently found in the muscle layer

Elimination of DSM from intestinal vessels depends on the separate processes of sphere degradation and wash out of polysaccharide fragments by the blood stream (ARFORS et coll FORSBERG 1978b) The degradation rate is reflected in the exponential elimination of DSM linked ^{99}Tc^{m} from the mesenteric vessels with an observed mean half time of 17 min Individual results (e g cat No 7) indicate that rapid elimination of DSM linked ^{99}Tc^{m} from the intestinal vessels (Fig 5) may be associated with early recovery of regional flow (Fig 1 Table 1)

For any degree of DSM induced arteriolar block DSM linked tracer will always be retained in the mesenteric vessels until microsphere degradation permits passage through the capillaries Blood flow can be measured by isotopic clearance methods if

the isotope is freely diffusible and does not itself influence regional flow (ZIERLER 1965) Neither condition applies to DSM linked $^{99}Tc^{m}$ clearance from the mesenteric vessels thus exact calculation of intestinal blood flow from gamma camera counts is not possible However provided a total or near total arteriolar obstruction is induced the rate of initial intestinal elimination may permit an estimate of the duration of the resulting intestinal ischaemia (Fig 6) Early elimination of DSM linked $^{99}Tc^{m}$ was associated with recovery of intestinal blood flow

After reaching the liver $^{99}Tc^{m}$ labelled mono di or poly saccharides or starch fragments may be retained in hepatic glycogen stores or phagocytosed by Kupffer cells in the liver sinusoids thus accounting for the observed rapid hepatic accumulation and excretion of activity (Fig 2b) A continuous $^{99}Tc^{m}$ leakage into and redistribution by the systemic circulation would also be expected The early marked deflection of he[illegible]ic activity from the straight line in the semil[illegible]mic plot (Fig 2b) and the increase in b[illegible]ctivity illustrated in Fig 6 confirm that su[illegible] leakage did occur The leakage of [illegible] systemic circulation together with possi[illegible] isotope excretion would also explain the [illegible]erved simultaneous hepatic decrease and intestinal increase of activity after 120 min

The present experiments differ from those previously reported concerning temporary regional ischaemia induced by DSM (ARFORS et coll FORSBERG 1978b FORSBERG et coll 1978a FORSBERG & JUNG 1978 FORSBERG et coll 1978b FORSBERG et coll 1979) in the following respects a) the detailed tissue flow determinations performed separately in the intestinal mucosa and muscularis along the length of the small intestine b) the experimental design using the gamma camera to obtain information about intestinal and hepatic degradation of $^{99}Tc^{m}$ labelled DSM c) the choice of the cat as animal model The results confirm and extend those of previous reports demonstrating that intra arterial DSM injection rapidly and consistently induces a temporary circulatory block followed by full recovery of regional blood flow While the regional use of vasoconstricting drugs to induce a similar degree of ischaemia may adversely affect the systemic circulation (FREEDMAN et coll 1978) the injections of DSM did not influence cardiac output

The temporary minor decline in renal blood flow following DSM injection was probably caused by DSM spill-over from the superior mesenteric artery into the aorta and subsequent DSM impaction in renal arterioles Such spill over into a blood ve[illegible] other than the injected one may confer hypo[illegible] radiation protection to tumour bearing tissue must be avoided

A plateau of minimum arterial flow lasting for 6 min was achieved in all animals Allowing a per[illegible] of about 4 min to deplete mucosal oxygen (ARFO[illegible] et coll) approximately 2 min are left for delivery [illegible] abdominal irradiation while the small intestine holds hypoxic radiation protection This is possi[illegible] with modern linear accelerators delivering 4 [illegible] min

In man DSM dosage adjustments to the amyl[illegible] activities met in human blood may be necessary [illegible] achieve a temporary flow obstruction of the desi[illegible] duration External detection of tracer activity [illegible]jected with the DSM may offer the clinician a se[illegible] quantitative non invasive assessment of mesente[illegible] blood flow and enable a selection of the appropri[illegible] time for radiation therapy

SUMMARY

Temporary ischaemia was induced in cat small intes[illegible] by degradable starch microspheres Regional arterial [illegible] tissue blood flow immediately fell by 85 per cent [illegible] subsequent normalization within 26 min after micro[illegible] injection Degraded $^{99}Tc^{m}$ labelled microspheres w[illegible] exponentially removed from intestinal vessels and [illegible]cumulated in the liver Provided near total arteri[illegible] obstruction is induced external isotope detection [illegible] permit assessment of the duration of the induced [illegible]chaemia which implies a selection of the appropriate t[illegible] for radiation therapy

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RADIATION THERAPY OF PRIMARY VAGINAL CARCINOMA

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Vaginal carcinoma is a rare tumor of elderly women It accounts for about 1 to 2 per cent of all gynecologic malignant tumors with a mean age at onset of between 60 and 65 years (CHAU 1963 PASQUIER 1970 MURPHY 1971 UNDERWOOD & SMITH 1971 PEREZ et coll 1973 PREMPREE et coll 1977 GERBAULET et coll 1978)

Mainly due to its rarity there is a considerable lack of information concerning this neoplasm Most reports describe a small number of cases collected over many years (DAW 1971 MARCUS et coll 1978) Moreover patients with carcinoma in situ and non epithelial tumor are sometimes grouped together with cases of primary invasive carcinoma as well as patients previously operated upon or irradiated for other gynecologic malignant tumors (BROWN et coll 1971 PEREZ et coll MARCUS et coll)

Vaginal carcinoma was previously considered to have a poor prognosis (FRICK et coll 1968) but improved results have been reported in the past 10 years (BROWN et coll MURPHY PREMPREE et coll MARCUS et coll)

It has not yet been assessed whether surgery or radiation is to be preferred as the method of therapy HERBST et coll (1970) DAW and UNDERWOOD & SMITH have reported series treated both with surgery and irradiation alone or in combination However no definite conclusion can be drawn as the series are not comparable

Surgery may be chosen in selected cases but often causes severe complications (HERBST et coll UNDERWOOD & SMITH) due to the extended procedure that is often required by the anatomic connections of the vagina (MARCUS et coll)

Radiation therapy has been shown to be effective (BROWN et coll PREMPREE et coll MARCUS et coll) but severe adverse effects may occur after irradiation with high doses (PEREZ et coll PREMPREE et coll)

The results now reported indicate that carcinoma of the vagina is possible to control with radiation therapy without significant adverse effects

Materials and Methods

The series consisted of 22 patients with primary vaginal carcinoma referred for radiation therapy to this institute from January 1965 to January 1975 The age ranged from 44 to 77 years with a maximum incidence between 70 and 75 years (8/22 i e 36.5%) mean age 65

In all cases the microscopic diagnosis was invasive squamous cell carcinoma The possibility that a vaginal localization of a primary arising elsewhere could have been diagnosed as a vaginal carcinoma was excluded for the following reasons the uterine cervix was intact in all cases except in 2 patients in whom hysterectomy had been performed 6 and 10 years before referral for uterine leiomyofibroma (microscopically proven) and the vulva urethra and anus were intact in all cases

Besides vaginal and general examination at refer

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ral chest radiography urography barium enema cysto and proctoscopy were performed in all cases

Each patient was retrospectively assigned to a clinical stage according to the 1961 International Federation of Gynaecology and Obstetrics (FIGO) classification Stage 0—Pre invasive carcinoma (carcinoma in situ) Stage I—Tumor limited to the vaginal wall Stage II—Tumor involving the sub vaginal tissue but without extension to the pelvic wall Stage III—Tumor extended to the pelvic wall Stage IV a—Tumor involving the mucosa of the bladder or rectum with or without extension beyond the true pelvis Stage IV b—Distant spread

Sixteen patients had a tumor stage I one a stage II 4 a stage III and one a stage IV a Inguinal meta static nodes were present in 3 patients (2 with a stage I and one with a stage III tumor)

Surgical procedures were performed only for biopsy of the primary tumor and of the inguinal nodes

A radical ir [illegible] n was planned in 18 patients (13 stage I on [illegible] 4 stage III) The treatment was only pall [illegible] remaining 4 patients (3 stage I one st [illegible]

All [illegible] radical treatment were ir radi [illegible] cobalt or 31 MV roentgen rays fron [illegible] through anterior and posterior opp [illegible] ncluding the pelvis and the vagina Inc [illegible] re included only if metastatic or if the [illegible] d the lower third of the vagina The m [illegible] orbed dose ranged from 50 to 60 Gy in 5 [illegible] ks (5×2 Gy per week)

A vaginal boost of a further 20 to 30 Gy to the mucosal surface with a radium loaded colpostat or a mould applicator for after loading technique (^{137}Cs or ^{192}Ir) was planned for each of the 13 patients with a tumor limited to the vagina and given radical ir radiation However this type of irradiation was giv en in 6 cases only It was not used in the remaining 7 patients due to a severe mucosal reaction of the vagina at the end of the external irradiation

In the palliative treatments volume and beam ar rangement varied according to the requirements of each clinical situation the dose never exceeded 40 Gy in 4 weeks

The condition of the patients was assessed at March 31 1980 The minimum follow up period was 5 years from the end of the irradiation no patient was lost to follow up

The therapeutic response has been evaluated in terms of local control of the neoplastic mass and of

Table 1

Complete disappearance and 5 year survival corre lated with the stage of the tumor (radically ir radiated patients) Number of patients with meta static inguinal nodes given in parentheses

Stage	Complete disappearance	5 year survival
I	11/13 (2/2)	8/13 (1/2)
II	0/1	0/1
III	2/4 (1/1)	2/4 (0/1)
Total	13/18	10/18
Per cent	72	55 5

Table 2

Complete disappearance and 5 year survival corre lated with the type of treatment (radically ir radiated patients stage I) Number of pa tients with metastatic inguinal nodes given in parentheses

Treatment	Complete disappearance	5 year survival
External irradia tion only	6/7 (2/2)	5/7 (1/2)
External and endo- vaginal irradiation	5/6	3/6
Total	11/13	8/13

5 year survival The adverse effects of the irrad tion have been classified as slight medium or vere

Results and Discussion

The local control of the disease was evalua within 2 months after the end of irradiation

Complete disappearance of the tumor w achieved in 13/16 patients (i e 72%) given rad irradiation In none of the 4 patients given palliat treatment was a complete objective response tained symptomatic relief was nevertheless attain in all four cases

The overall 5 year survival amounts to about per cent In the radically irradiated patients a 5 ye survival rate of 10/18 (i e 55 5%) was achieved patient given palliative irradiation survived for mo than one year All patients died because of the d ease having a local persistent or recurrent neopla tic mass

Table 3

Five year survival rate after radiation therapy as reported in the past 10 years

Authors	Year	Stage distribution and survival				Overall survival
		I	II	III	IV	
BROWN et coll	1971	11/16	13/19	4/15	0/11	8/61 (46)
GERBAULET et coll	1977	–	–	–	–	17/5 (30)
PREMPREE et coll	1977	5/6	20/31	8/20	0/7	[illegible] (5)
MARCUS et coll	1978	4/5	?/4	1/1	1/?	[illegible] (67%)

The rate of complete disappearance and of 5 year survival correlated with the stage of the disease appears in Table 1

Complete disappearance and 5 year survival in correlation with the type of treatment (i e external irradiation alone versus external irradiation plus endovaginal irradiation) were analysed only for patients of stage I The results appear in Table 2

Adverse effects Effects not lasting for more than 3 months and consisting of mucosal and cutaneous reactions diarrhea and acute cystitis were classified as slight They occurred in 15 patients and disappeared after medical treatment

Effects lasting for more than 3 months but controlled by medical treatment were classed as being of medium grade They consisted of mucosal and cutaneous chronic abnormalities and hemorrhagic proctitis or cystitis and were observed in 3 patients

Severe adverse effects *requiring surgical treatment* such as stenosis necrosis or fistulae did not occur in any patient

Radiation therapy of primary vaginal carcinoma has been reported to be successful both in terms of complete disappearance of the disease (91% MARCUS et coll) and of survival (5 year survival rates as reported in the literature are listed in Table 3)

The present results with 72 per cent complete disappearance and 55 5 per cent 5 year survival for radically irradiated patients confirm that vaginal carcinoma can be cured by radiation therapy

The present series indicates that a good result can be achieved with radical irradiation particularly in the early stages of the disease (8/13 i e 61 5% year survival rate in patients stage I) and that an endovaginal boost dose does not seem to improve the results of external irradiation (3/6 versus 5/7 year survival rate)

Adverse effects of irradiation are reported to be frequent particularly for treatments with high doses such as a combination of external endocavitary and interstitial irradiation with about 10 per cent severe complications (PEREZ et coll PREMPREE et coll) medium or slight adverse effects have not been specified in any of the reviewed reports

The adverse effects are low in the present series no complication required surgery and only 3 cases had adverse effects of a medium grade

The present data indicate that radiation therapy can be adopted in many cases of primary carcinoma of the vagina with effectiveness and safety This treatment is appropriate particularly for elderly patients who are in the majority with this type of tumor

SUMMARY

In a series of 22 patients with primary invasive squamous cell carcinoma of the vagina (stage I through IV a) a radical irradiation was planned in 18 and a palliative in the remaining 4 patients The 5 year survival rate in the radically irradiated patients was 10/18 for all stages and 8/13 for patients of stage I A vaginal boost irradiation did not seem to improve the results of external irradiation in patients of stage I Severe adverse effects did not occur

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RELATIONSHIP BETWEEN HISTOLOGIC GRADING OF HEAD AND NECK TUMOURS AND REGRESSION AFTER CHEMOTHERAPY

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In 1973 JAKOBSSON reported on the relationship be ween histologic grading and recurrence of laryngeal carcinoma after irradiation Using a mul factorial grading system he found a significant cor lation in a series of 240 patients LUND et coll (5 a b 1976 1977) modified the system and used t on series of carcinoma of the lip tongue and larynx They also found a significant relationship between histologic grading and the clinical result of treatment However the only practical consequence has been a suggestion of more frequent follow ups It is likely that a certain histologic grade is correlated to the effect of certain schedules of treatment with cytostatic drugs The present investigation was per formed to elucidate this hypothesis

Material The series comprised 43 consecutive patients with 44 tumours of the head and neck (one patient had 2 tumours) all treated during the period Jan 1 1977 to Jan 1 1979 (Table 1) More than half of the cases had lymph node metastases in the neck on admission (Table 2) Two cases belonged to stage 2 (TNM-classification UICC 1978) 23 to stage 3 and 16 to stage 4 In 3 cases no staging was obtained and no case belonged to stage I The sex and age distribution appears in the Figure All patients were given BMV-chemotherapy before irradiation the doses were calculated from the surface of the pa tients Bleomycin 13 mg/m² intramuscularly Methotrexate 70 mg/m² intravenously and Vincris tine 1 mg/m² intravenously

In order to obtain the best effect the drugs were administered according to the schedule given in Table 3 Before the beginning of the course and on day 12 the tumour shrinkage was evaluated and expressed in per cent except in 3 cases where the evaluation was impossible because of the location of the tumours Immediately after the chemotherapeutic course treatment with ^{60}Co was started The aim was to administer 57 to 60 Gy during 6 weeks with 5 irradiations weekly using opposing fields both irradiated each day Details on the irradiation will be found in the report by JØRGENSEN & SELL (1971)

The histologic grading was performed on the primary biopsy material using the rules modified by LUND et coll (1977) The distribution of the cases according to the histologic score is given in Table 4

Results

The results of the analysis appear in Table 5 The tumour shrinkage is expressed in per cent in 5 groups and the mean histologic score was calculated in each group It is evident that no relationship between the tumour shrinkage and the histologic score existed Independent of the method of analysis the result was the same

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Table 1

Location of primary tumour

Location	No of tumours
Oral cavity	6
Rhinopharynx	6
Oropharynx	12
Hypopharynx	4
Supraglottis	4
Glottis	9
Maxillary sinus	3
Total	44

Table 2

TNM classification (UICC 1978)

	No	N1	N2	N3
T1a	–	1	–	1
T1b	–	3	–	
T2	2	2	1	2
T3	11	4	1	6
T4	3	3	–	1
Maxillary sinus	3	–	–	–
Total	19	13	2	10

Table 3

Schedule for treatment with cytostatic drugs (BLM = ble[illegible] MTX = methotrexate VCR = vincristine)

	Day			
	1	5	8	1[illegible]
8 a m	VCR		VCR	
1 p m	BLM+MTX	BLM	BLM+MTX	BL[illegible]

Table 4

Distribution of tumours in the different histologic scores

Score	No of tumours
1 50–1 99	2
2 00–2 49	9
2 50–2 99	14
3 00–3 49	14
3 50–4 00	5

Table 5

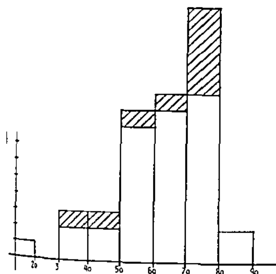
Relationship between tumour shrinkage after chemotherapy and histologic score

Shrinkage (per cent)	No of tumours	Mean histologic score
0–20	7	[illegible]98 } 19– [illegible]
21–40	3	[illegible]49
41–60	9	[illegible]90
61–80	8	[illegible]65 } 2[illegible]
81–100	14	[illegible] 77
No evaluation	3	–

Discussion

Previous publications concerning the relationship between histologic grading and clinical results of treatment have shown a significant correlation between histologic grading and recurrence of the disease (JAKOBSSON LUND et coll 1975a b 1976 1977 WILLÉN et coll 1975) Possibly a relationship between histologic grading and tumour response after chemotherapy exists Certain cytostatic drugs may influence tumours of a certain histologic grade The drug[illegible]sed in the present series evidently had no effect [illegible]he tumours A possible explanation is that th[illegible] drugs attack in a complex way in the life [illegible]he malignant cell (O CONNOR et coll 197[illegible]atment schedule (Table 3) was based on [illegible]hat vincristine damages cells which are in [illegible] or premitotic phase resulting in an accumulation of mitosis 6 to 8 hours later bleomycin is most injurious in the mitotic and G phase and methotrexate has its greatest effect in the S phase This complexity of the chemotherapeutic effects possibly does not permit any clear evaluation of the relationship between the histologic grading and tumour response All previously published series except the one by HELWEG LARSEN et coll (1978) suggest a correlation between the clinical results and histologic grading Therefore in the future the histologic grading must be correlated to different principles of treatment For the time being in this hospital it is planned to treat patients with epidermoid carcinomas with radiation sensitizers and correlate the results with the histologic grading

Possibly a revision of the grading system itself by computer multifactorial analysis is motivated i e



...ge distribution at the time of histologic diagnosis Hatched ...s indicate females (n=9) white columns males (n=34)

...lusion of one or more less relevant parameters ...s used and instead include other factors WIL...N et coll actually reduced the system to 6 factors ...luding the parameters structure and vascular ...sion Using this modification WILLEN et coll ...und a clear correlation in a series of 124 patients ...ith gingival carcinoma Epidermoid carcinoma con...ns blood type iso antigens in quantities depending ...their stage of differentiation (DABELSTEN et coll ...74) this fact may be a valuable supplement to the ...ologic grading Other parameters such as the ...tent of DNA (JAKOBSSON et coll 1975) might ...o be useful and PAAVOLAINEN et coll (1973) have ...en a relationship between the grade of malig... ...y and content of mucopolysaccharides of the ...oma of epidermoid carcinoma

Conclusion Histologic grading has been reported ...give a valuable supplementary information to the ...ical evaluation of patients with head and neck ...ours However in the present series of 44 ...ours no correlation was found between the effect ...combined BMV treatment and histologic grading ...ther testing of the grading system with other ...rapeutic regimes seems important and possibly ...o attempts to revise the system

SUMMARY

A series of 43 patients with 44 primary head and neck tumours were treated by combined BMV chemotherapy (bleomycin methotrexate and vincristine) given before irradiation The tumour shrinkages were evaluated and related to the histologic grading of the primary biopsy material No relationship was found between the tumour response and degree of malignancy as evaluated by the histologic grading The clinical relevance and possible modification of the histologic grading system are discussed

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EFFECTS OF ACUTE GAMMA IRRADIATION ON SPERMATOGENESIS AS REVEALED BY FLOW CYTOMETRY

U HACKER J SCHUMANN and W GOHDE

A method rapidly revealing injury caused by ir ...ation or chemical compounds in vivo will be of ...a importance to ascertain cytotoxic and mu ...enic effects The cytofluorometric analysis pro ...des such a method for measuring the amount of DNA in single suspended cells with a high degree of accuracy (MEISTRICH et coll 1978 a GÖHDE et coll 1979 a)

The DNA distribution is related to the number of ...erent cell types in a given tissue In the testis this distribution reveals the number of cells in the ...fferent stages of spermatogenesis Using cyto...ometry of irradiated testes alterations of the ...A-content of single cells should be possible to ...onstrate i e changes in the cell kinetics of sper...ogenesis increase or decrease of the frequency ...fferent cell types and mutations ...lterations of the cell cycle and the cell phase ...ression of different cell types induced by differ... kinds of radiation have previously been demon...ed by pulse cytophotometry (GÖHDE 1973 ...O & GÖHDE 1976) but not the effects of gamma ...on the phase progression cell differentiation ...nduction of genetical aberrations of mammalian ...s cells

Material and Methods

...le mice of the inbred strain NMRI aged 8 to 13 ...s were used

...adiation Before the irradiation the mice were anaesthetized by Evipan given intraperitoneally The testes were then irradiated locally with 0 1 0 25 or 0 5 Gy at a dose rate of 1 873 Gy/min or 1 2 5 5 10 or 15 Gy at a dose rate of 4 125 Gy/min using a ^{60}Co-source During the exposure the whole body with the exception of the testis region was shielded with lead At doses below 1 0 Gy 4 experimental and 4 control mice were killed per dose and time point at doses of 1 0 Gy and above at least 2 irradiated and 2 control mice were analysed

Preparation of cell suspension The testes were removed from fat and connective tissue Both testes from each animal were minced using surgical blades and stirred 5 to 8 min in a pepsin solution (Pepsin Serva 130 Anson units/mg pH 1 8) on a magnetic stirrer at room temperature (ZANTE et coll 1976 ZANTE et coll 1977) This preparation method results in a real single-cell suspension The cells were neither washed nor centrifuged thus selective cell loss was prevented

Staining of DNA The DNA of the cells was stained either with a dye solution containing 5 μg/ml ethidium bromide (Serva Heidelberg) 12 5 μg/ml mithramycin (Serva Heidelberg) and 1 5 mg/ml MgCl in tris buffer pH 7 4 (ZANTE et coll 1976 1977) or with a DAPI dye solution (2 5 μg/ml DAPI (4 6-Diamidino 2 phenylindol 2 HCl) in tris buffer pH 8 0 containing 0 5 mol MgCl GÖHDE et coll 1979 b) Both dyes result in identical fluorescence distributions (GÖHDE et coll 1979 b)

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Flow cytometry Measurements were performed with the new pulse cytophotometer developed by GÖHDE including the glass laminar flow chamber (GÖHDE et coll 1979 a)

DNA histograms As reported in detail by ZANTE et coll (1977) the DNA fluorescence distributions of testicular cells obtained by the pulse cytophotometer are characterized by four peaks (Fig 3 a) Peak III represents 2c (diploid) G_1 spermatogonia secondary spermatocytes Leydig cells and macrophages This group normally comprises 12 to 17 per cent of a testis cell suspension Peak IV is formed by cells with a 4c DNA content primary spermatocytes spermatogonia and probably some interstitial cells in the (G_2+M) phase This peak normally contains 10 to 13 per cent of the testis cell suspension

The S phase cells (spermatogonia preleptotene spermatocytes other S phase cells) between the 2c and 4c peak constitute 3 to 7 per cent of the testis cells Two peaks with 1c (haploid) germ cells must be discriminated round spermatids are represented in Peak II They stain stoichiometrically with the 2c and 4c-cells and form about 40 per cent of the testis cells Cells which are stored in Peak I show without a special enzymatic treatment about 70 per cent of the fluorescence intensity of cells registered in Peak II (ZANTE et coll 1977) Peak I represents elongated spermatids and spermatozoa (ZANTE et coll 1977) with the normal haploid DNA content Their non proportional DNA staining is due to the highly condensed chromatin (MONESI 1964 1965) These cells normally comprise 24 to 32 per cent of a testicular cell suspension of an adult fertile mouse

The c S and 4c percentages were computed with the Interactive Spectrum Analyzing Program using a PAS Multichannel Analyzer and a PDP 11 computer (FRUH & GOLDING 1976) The percentages of the two groups of 1c cells were estimated by computing the area under each peak Significance analysis was performed with the t test Each analysed histogram consisted of about 50 000 measured cells

Results

Irradiation heavily interferes with spermatogenesis as demonstrated by characteristic changes of the DNA distribution

The alterations of the 2c S 4c and 1c-cell frequencies after acute gamma irradiation with 5 Gy appear in Fig 1 At least two experimental and two

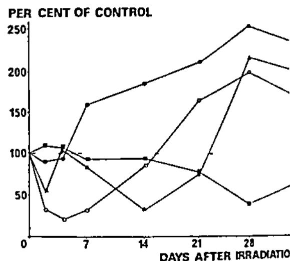


Fig 1 Alterations of the percentages of the 2c (●) S (O) 4c and the sum of the 1c-cells (■) of mouse testis at different intervals (2 to 35 days) after irradiation with 5 Gy in per cent control

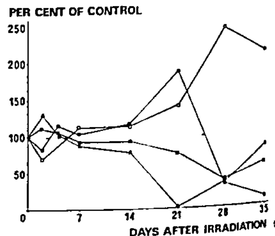
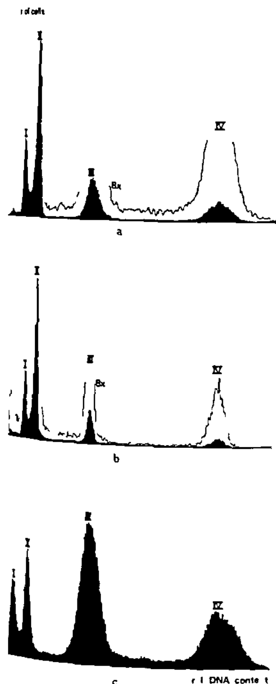


Fig 2 Alterations of the percentages of round spermatids (elongated spermatids and sperm (●) the sum of both cell-ty (■) and the total number of the c S- and 4c-cells (O) of mo testis at different intervals (2 to 35 days) after irradiation wit Gy in per cent of control

control animals were killed on day 2 4 7 14 21 and 35 after irradiation The average of the d from irradiated mice was expressed in per cent the mean control figure The total percentage the haploid germ-cells (peak I and II of the DNA h tograms) is given in the same figure The most ob ous effect shortly after irradiation was the i mediate decrease of the number of S phase cells about 1/5 of the control value during the first t days after the exposure (Fig 3 b) At the sa time the percentages of the 2c and 4 -cells we reduced to about 90 and 50 per cent respectively the controls



DNA distributions of mouse testis cells Abscissa Relative fluorescence values 256 channels Ordinata Number of [illegible] cells per channel a) Control mouse CV(II)=3 5% [illegible] of measured cells 50000 b) Mouse 2 days previously [illegible] with 5 Gy CV(II)=3% CV(III)=2 2% Number of [illegible] cells 30000 c) Mouse 28 days previously irradiated [illegible] Gy CV(II)=6 3% Number of measured cells 150000

[illegible] the 2c-cells and the cells in proliferation (S [illegible] were reduced the relative number of the haploid cells increased This indicates that the more [illegible] spermatids (1c-cells) are less sensitive [illegible] the more primitive forms of spermatogenesis (2c S 4c cells) After the initial decrease the percentage of 2c cells increased and reached its highest point 28 days after the [illegible] (2 5 times the control values) This [illegible] increase was initially due to the decrease [illegible] 4c-cells and after the 14th day due to the [illegible] haploid germ-cells

The number of S [illegible] lls reached its lowest value (20% of con[illegible] after the irradiation It remained constant [illegible] the 7th day after irradiation and then [illegible] d until it reached a maximum of abo[illegible] h control value 28 days after the irradiation [illegible] increase of the relative amount of S [illegible] recovery processes and is partially due [illegible] the decrease of 1c-cells at the same time The [illegible] of 4c-cells increased above the control [illegible] 4 days after irradiation It subsequently declined until it reached a minimum value (30% of control) 4 days after the exposure This decrease of 4c cells (mainly primary spermatocytes) is caused by the preceding killing of spermatogonia Thereafter the number of 4c-cells increased again until it reached its highest point (about 200% of the control value) 28 days after the irradiation

The alterations of the haploid germ-cells (cells of Peak I and II) that occur after irradiation with 5 Gy appear in Fig 2 The total of the 2c S and 4c cells is also shown in the same figure After 2 days and after 4 days the total percentage of haploid cells had increased significantly ($p<0.05$) but none of the special haploid cell types was significantly altered during the first week after irradiation From the 7th day after the exposure the percentage of sperm and elongated spermatids decreased below the control value and reached its lowest point 21 days after the irradiation According to the time table of OAKBERG (1957) the elongated spermatids and sperm were spermatids 7 days earlier spermatids and primary spermatocytes in late meiosis 14 days earlier and primary spermatocytes in early meiosis 21 days earlier The round spermatids are reduced from the 7th day after exposure and disappear nearly completely after about 3 weeks At the same time the 1c-cells are reduced to 75 per cent This is a consequence of the sharp reduction of round spermatids irradiated as more mature spermatogonia which are extremely radiation sensitive (OAKBERG 1955 b)

One week later the gap of missing cell types had shifted somewhat and involved elongated spermatids and sperm which were reduced to 1/3 of the control At the same time the total percentage of

haploid germ cells reached the lowest value (35% of control) while the number of round spermatids increased again In contrast to the reduction of the haploid germ-cells the percentage of 2c S and 4c cells had relatively increased above the control value

The percentage of round spermatids along with that of all haploid germ-cells had 35 days after the irradiation increased while the number of elongated spermatids and sperm had further declined to 10 per cent of controls as a consequence of maturation depletion due to killed spermatogonia (Examples of the radiation induced alterations described appear in Fig 3) The control histogram has the following distribution elongated spermatids and sperm 27 per cent round spermatids 38 2c cells 17 S phase 6 and 4c cells 12 per cent The coefficient of variation of Peak I is 5 5 per cent of Peak II 3 5 of Peak III 4 7 and of Peak IV 3 5 per cent The coefficient of variation (CV) of Peak I and Peak III are normally broader than that of Peak II probably because Peak II is composed of only one subfraction (round spermatids) whereas the other two peaks represent cells with a different stainability of the nuclear material Peak I contains elongated spermatids and sperm and Peak III is composed of 2c spermatogonia in different phases of maturation secondary spermatocytes and some other cells (macrophages Sertoli cells Leydig cells)

A histogram of the testes of a mouse 2 days after irradiation with 5 Gy is illustrated in Fig 3b The most obvious effects are the reduction of S phase (the upper line is multiplied by a factor of 8) to 1 2 per cent the reduction of the 4c Peak to 6 per cent and the extreme narrow form of Peak III which has a CV of only 2 2 while Peak II has a CV of 3 per cent The reduction of the CV of Peak III was also observed after other radiation doses and may be a consequence of killing of one (or several) subfraction(s) of this peak possibly the highly sensitive differentiated spermatogonia

The situation 28 days after the irradiation is presented in Fig 3c Most obvious is the remarkable increase of Peak III (2c-cells) to 40 per cent which was caused by a relative increase of interstitial and Sertoli cells due to the sharp reduction of haploid germ-cells (Peaks I and II) The increase of Peak IV (4c-cells) and in the S region was less marked than that of the 2c Peak At this time the testes were atrophic and the weight was reduced to 20 per cent of the control value

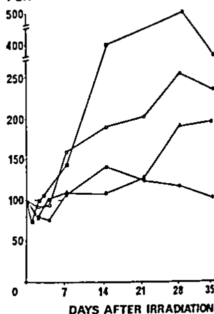


Fig 4 Alterations of the percentage of 2c-cells after irradia[tion] with 1 (●) 2 5 (▲) 5 (O) and 10 (■) Gy in per cent of contr[ol at] different intervals (? to 35 days) after the irradiation

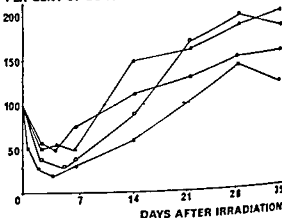
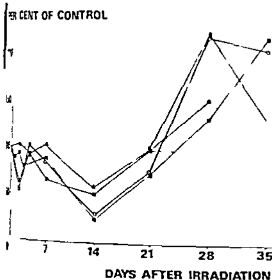


Fig 5 Alterations of the percentage of S-phase cells after irra[dia]tion with 1 (●) 2 5 (▲) 5 (O) and 10 (■) Gy in per cent of con[trol] at different intervals (? to 35 days) after the irradiation

The number of 2c-cells after irradiation with d[if]ferent doses are demonstrated in Fig 4 Follow[ing] irradiation the 2c-cells initially and independen[t] of the dose decreased due to killing of sp[er]matogenic cells (spermatogonia) Several days la[ter] the number of 2c cells increased in a dose depe[nd]ent manner with a maximum value of 5 times t[he] control value 28 days after exposure The incre[ase] of 2c-cells was accompanied by a dose-depend[ent] reduction of the testis weight and is mostly due to [a] relative increase of interstitial and Sertoli ce[lls]



6 Alterations of the percentage of 4c-cells after irradiation 1 (●) 7.5 (▲) 5 (O) and 10 (■) Gy in per cent of control

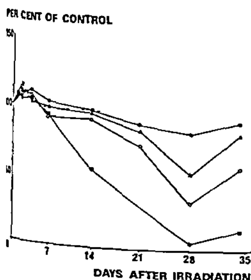


Fig 7a

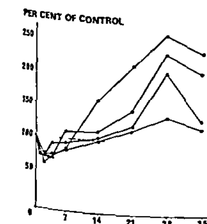


Fig 7b

Alterations of the percentage of a) haploid cells and b) c c-cells after irradiation with 1 (●) 5 (▲) 5 (O) and 10 in per cent of control

(ITAGAKI 1970) This corre p nds to the shrinkage of the seminiferous tubul a con equence of the reduction of germ cells (I 1)

The change of the S ph cell frequencies after irradiation appear in F The percentage of S phase decreased dose de ndently during the first days The reco e o dose-dependent but reduced at the hi ed

The percentag ch fter irradiation is presented in Fig 6 ell frequencies appear to be nearly independe ne dose After some fluctuations minimum v occurred after 14 days These minimum values due to killed mature spermatogonia which to a sharp reduction of primary spermatocyt Thereafter the percentage of 4c cells increased hed a highest value 28 days after irradiation wi ses below 10 Gy and then declined again At irradiation with 10 Gy the number of 4c cells still increasing 35 days later indicating that reco ery processes after this high dose have not yet reached the same level as compared with the lower doses

The total 1c-cell frequency of the testes after irradiation appears in Fig 7a and those of the sum of the 2c S and 4c-cells in Fig 7b Soon after the exposure all haploid cells increased relatively which was caused by the decrease of the 2c S and 4c-cells None of the effects had any strong dose dependency Between the 4th and the 14th day until the 28th day the 1c-cell frequency decreased below the control value while the sum of the 2c S and 4c-cells raised above the control value Both effects were dose dependent The first effect is a consequence of the reduction of spermatids irradiated as primary spermatocytes and spermatogonia The second effect is initially caused by an increase of 2c-cells (relative increase of interstitial cells) and later by an increase of S and then 4c-cells (recovery processes and relative increase due to killing of germ-cells) Both effects are a consequence of the state of the testes the more mature haploid germ cells which were in the stage of spermatogonia at the time of irradiation lead to a temporary depletion of the seminiferous epithelium with germ-cells The 1c-cell proportion increased again 35 days after the irradiation and the total of the 2c S and 4c-cells decreased These dose-dependent effects indicate that the repopulation and the recovery processes already passed their maximum and that normalization was continuing

A DNA histogram obtained 21 days after an ir

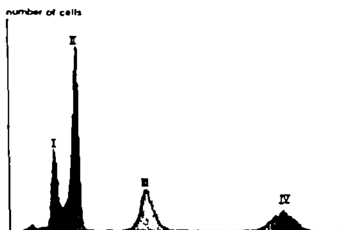


Fig 8a

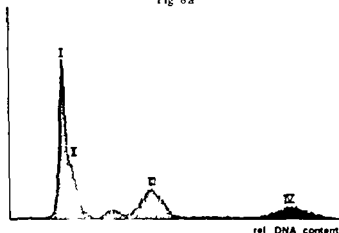


Fig 8b

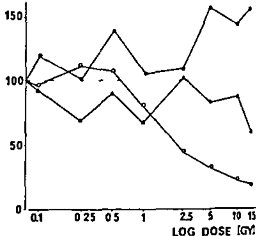


Fig 9

Fig 8 DNA-distributions of mouse testis cells a) Control m Number of measured cells 50 000 b) Mouse 21 days prev irradiated with 2 5 Gy Number of measured cell 30 000

Fig 9 Alterations of the percentages of the 2c (●) S (○) 4c-cells (▲) 7 days after irradiation with different doses (0 1 Gy) in per cent of control

radi Gy is illustrated in Fig 8b It is n the extra peak between the 1c and th region represents It shows two times the modal channel number of Peak I but is very broad (high coefficient of variation) If this peak would be caused by clumped cells the broadness must be nearly the same as in the other peaks

Fluorescence microscopy revealed that these cell suspensions contained abnormal oversized elongated spermatids and spermatozoa with two heads corresponding to the extra peak Extra peaks were also observed in histograms of testis cells irradiated 14 days earlier Cells that form elongated spermatids or sperm 14 or 21 days after irradiation were exposed as primary spermatocytes which tend to form abnormal spermatids and sperm (OAKBERG & DI MINNO 1960) Round spermatids (Peak II) which were irradiated as mature spermatogonia had decreased to about 10 per cent (control more than 40%)

Another point of interest is the increase in the CV of the peaks in the histogram after irradiation indicating changes in the chromatin structure and possibly also minor chromosome aberrations (Figs 3c)

The frequencies of the 2c S and 4c-cells at 7th day after irradiation with different doses rang from 0 1 to 15 Gy are demonstrated in Fig 9 A doses below 1 Gy 4 experimental and 4 control m were killed after doses of 1 Gy and above onl experimental and 2 control mice were analysed each dose The percentage of the 2c-cells was ra above the control value after all doses Even a 0 1 Gy a significant ($p<0.05$) relative increase 2c cells occurred This is a consequence of the d inution of S phase and 4c cells The percentage S phase cells was slightly reduced after 0 1 Gy increased after 0 25 and 0 5 Gy After higher d the frequency of S phase cells diminished in a d related manner This decrease does not show a si ple exponential function It is caused by seve precursors of the cells now constituting S pha e more radiation resistant A_1 stem cells (LD_{50} 2-3 and A_{pr} cells and the more radiation sensitive (LD 1 Gy) and A_1 spermatogonia (LD 0 ... MEISTRICH et coll 1978a OAKBERG 1971)

he percentage of the 4c-cells was reduced under control value after all doses except 2 5 Gy

Discussion

veral kinds of radiation effects that express selves as alterations of the DNA distribution of estis cell suspension were detected

decrease of 2c-cells shortly after irradiation was ed mainly by killing of differentiated 2c sper gonia which are extremely sensitive in respect diation induced cell-death (OAKBERG 1955a b) same time a drastic decrease of S phase cells observed caused by the killing of sper gonia This may be concluded from the fact he minimum value of S phase was observed at ame time as a minimum value of spermatogonia TWIG 1939 OAKBERG 1955a b) A decrease of ular S phase cells after irradiation has also observed by BRYAN & GOWEN (1956) MONESI and GUPTA & BAWA (1975)

hird effect appearing shortly after irradiation decrease of the 4c cell frequency which was a quence of the cell killing of spermatogonia and y spermatocytes No block in the G phase as er cell systems after irradiation was detected in cells

reduction of these three cell types (2c S 4c) after the irradiation led to a temporary rela crease of the haploid germ cells which had rradiated as spermatids or primary spermato n late meiosis when they were more resistant ation than most other testicular cells (OAK 955a b OAKBERG & DI MINNO) The initial on of 2c S- and 4c cells led to a dose de nt depletion of the seminiferous epithelium aploid germ cells At first 2c spermatogonia sed then the S phase cells 4c cells round tids and finally 28 days after the irradiation mber of elongated spermatids and sper a diminished (The S phase proportion is form d by spermatogonia and only to a small ion of preleptotene spermatocytes whereas compartment is mainly composed of primary tocytes and only to a lower degree of -spermatogonia) Thus the most obvious ons of the DNA-distribution as revealed by tometric analysis were caused by cells ir l as spermatogonia These cells normally die after irradiation in late interphase or mitotic e (OAKBERG 1955a)

Besides proliferation a teristic DNA dis tribution analysis also de ts other radiation in duced effects Cells irrad d in the stage of pri mary spermatoc tes do no ually die immediately after the irradi tion but d to form abnormal spermatids (OA BER & DI MINNO) Obviously spermatids with ir l DNA content (with diploid DNA-co tic aberrations that are reflected by ncre of the CV) can be detected by flow tric analysis

In addition the n of he CV of the peaks of DNA histogram i port nt information Thus the narrowing of peak two days after irradia tion reflects killin e matogonia The broaden ing of the peaks at di tion showed that muta tional changes of t NA occurred These can be detected by flow c etry only if the cells have divided

Regeneration proc es appeared after all doses At first the percentag f the S phase cells increased followed by an incre e of 4c cells beginning ap proximately one week later The percentage of hap loid germ cells 35 days fter the irradiation showed a dose-dependent increase following all doses

The disturbance of the testicular cytology after irradiation analysed with automated flow cyto- metric DNA estimation in single cells is in good agreement with the results of other much more time consuming methods (HERTWIG OAKBERG 1955a b)

The results show that the mouse spermatogenesis could be used as a biologic dosimeter some of the cell stages have a relatively high sensitivity to radia tion also in this flow cytometry test Further investigations about the dose-dependent induction of changes of the maturation of spermatozoa phase progression delay as well as mutagenesis (which is proportional to the increase of the CV of divided cells after irradiation) must be performed to estab lish the lowest dose detectable with the present technique Nevertheless the results indicate that this dose may be less than 0 1 Gy

The analysis of radiation induced mutations can be a further application of flow cytometry For this purpose it would be interesting to improve the resolution of the DNA measurements below 1 per cent of CV This is only partially a technical problem because on homogeneous human and ani mal cell populations the accuracy of DNA meas urements reaches CV values below 1 per cent (GÖHDE et coll 1979a) In the meantime the accu

racy of the sperm DNA analysis with the pulse cytophotometer could be improved measurements with separated peaks for X- and Y-chromosome bearing spermatids and sperm are now possible (MEISTRICH et coll 1978a OTTO et coll 1979)

SUMMARY

Mice irradiated with doses ranging from 0 1 to 15 Gy using a Co source and controls were killed at intervals varying from 2 to 35 days after irradiation The DNA content of the testicular cells in single cell suspensions was measured with the pulse cytophotometer to determine the frequencies of the different stages in the spermatogenesis The relative amount of S phase and 4c cells was reduced initially but increased subsequently to hypernormal values A decrease of 2c cells indicated a higher cell kill of diploid spermatogonia Gamma ray induced spermatids with abnormal DNA values (diploid sperm) were identified The results suggest that the spermatogenesis can be analysed with flow cytometry and used as a biologic dosimeter even for small doses of ionizing radiation

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FROM THE INSTITUTE OF RADIATION PATHOLOGY (DIRECTOR PROF H B LAMBERTS) UNIVERSITY OF GRONINGEN THE NETHERLANDS

UPTAKE OF SEROTONIN LIBERATED BY RADIATION OF RABBITS AND MICE

TJEERD VENINGA and WILLY LEMSTRA

Since about 1960 considerable interest has been directed to radiation induced alterations in the content of endogenous compounds of several organs, one of these being 5 hydroxytryptamine (5 HT serotonin) (RENSON & FISCHER 1959 MELCHING et coll 1960 WILLOUGHBY 1960 RANDIĆ et coll 1962 ERSHOF & GAL 1961 ERSHOF et coll 1962 BENNEMAN & VENINGA 1962 DEANOVIĆ et coll 1963 MATSUOKA et coll 1966 SMITH & LANGLANDS 1966 PENTILLA & KORMANO 1971) This hormone if free in the circulation is physiologically active and in some instances it may take part in pathologic processes Normally it is for the greater part stored in a particulate fraction of cells like neurons enterochromaffin cells and blood platelets (GARATTINI & VALZELLI 1965) Previously it was reported that in rabbits and mice irradiation causes an immediate increase of plasma 5 HT originating from the intestinal tract (VENINGA et coll 1973) Since that time the interest in bio release processes by irradiation appeared to have declined probably because the reaction was thought to have no serious consequences Apart from that 5 HT has been suggested as a putative biochemical indicator of radiation injury (DEANOVIĆ 1971 STREFFER 1971 VENINGA 1971) However as it seems of interest to know whether 5 HT is of real significance for this reaction a series of experiments with different radiation doses was carried out in order to analyse 5 HT release in association with interfering processes

Materials and Methods

Chinchilla rabbits and institute inbred grey mice have been irradiated under conditions as described previously (VENINGA et coll) Rabbits received an abdominal exposure whereas mice received either a total body or an abdominal irradiation In rabbits 3 ml aliquots of blood were collected from an ear vein 30 and 5 min before and 5 min after irradiation Murine blood was taken by orbital extraction immediately after the irradiation EDTA was used as anti coagulant Non irradiated animals served as controls The 5 HT level of plasma was determined by the method of ASHCROFT et coll (1964)

All animals were intraperitoneally injected 90 min before irradiation with 10 mg/kg iproniazide in order to prevent 5 HT break down A 10 cm piece of the small intestine 10 cm distal to the stomach was employed for determination of the amount of 5 HT in the intestinal lumen in mice The piece was excised immediately after irradiation and the lumen rinsed with 1 0 ml saline with the aid of a syringe The saline was collected in a Petri dish which after emptying was rinsed with another 1 0 ml saline 5 HT in the saline was determined according to the method described by BARCHAS et coll (1972) The mice were deprived of usual food for 48 h and drinking water for 36 h to omit contamination with the normal intestinal content Instead they received 24

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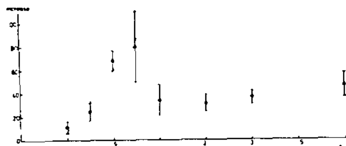


Fig 1 Serotonin increments in the blood plasma of rabbits after different doses delivered to the abdomen Values are expressed in percentages of the non irradiated controls (±SE)

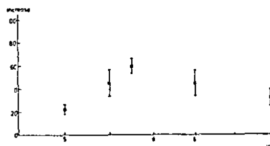


Fig 2 Serotonin increments in blood plasma of mice after di[illegible] ent doses delivered to the abdomen Values are expresse[illegible] percentages of the non irradiated controls (±SE)

h before irradiation a solution containing 1 0 per cent $MgSO_4$ and 10 per cent sucrose Intestinal pieces which nevertheless contained remainders of general food were discarded

For the determination of 5 HT in murine spleen the organs were homogenized in ice cold saline (1 25) with an Ultraturrax (Janke und Kunkel Staufen W Germany) 5 HT was measured in the clear supernatant using the method of ASHCROFT et coll

Results

In [illegible] and mice irradiation of either the total [illegible] abdominal region gave rise to in[illegible] [illegible] HT levels In both animal species a line[illegible] [illegible] response relationship existed over a range of roughly 0 75 Gy starting in rabbits with a dose of 1 Gy in mice with a dose of 5 Gy Thereafter lower values for plasma 5 HT were found (Figs 1 2) In a recent experiment with mice similar data were found except that the percentage 5 HT increase was still higher up to 90 per cent for 5 75 Gy thereafter lower values were observed again In contrast to the other experiments this last experiment was carried out with female mice

Doses below those indicated in the figures remained without effect No 5 HT was detected in the intestinal lumen of mice after irradiation with doses up to 7 50 Gy In contrast abdominal irradiation of mice resulted in a rise of 5 HT in the spleen (Table)

Discussion

The radiation induced release of 5 HT from the intestinal tract into the circulation was shown to be dose dependent but within a limited range Rabbits and mice differed in their sensitivity for 5 HT release the rabbits being much more sensitive Radia[illegible]

Table

Content of 5 HT in spleen of mice under normal conditions as well as after exposure to different doses of radiation The or[illegible] were excised immediately after exposure

Dose (Gy)	No of mice	5 HT (µg/g spleen ±SE)	Per cent rise (versus control)	p-value (versus control)
0 (control)	17	10 1+0 [illegible]		
5 5	1[illegible]	1[illegible] 8+0 6	[illegible]	<0 00[illegible]
6 5	15	1[illegible] 3±0 3	[illegible]	<0 00[illegible]
7 5	18	13 [illegible]+0 5	31	<0 00[illegible]

tion doses above those causing a linear response [illegible] to lower plasma 5 HT levels as compared with d[illegible] in the linear range These lower levels can [illegible] explained on the basis of a second process whic[illegible] increasingly mobilized by higher doses such as a[illegible] mentation of 5 HT metabolism or 5 HT uptake [illegible] increased permeability of the intestinal wall due [illegible] radiation injury

Augmentation of 5 HT metabolism is not [illegible] likely since the metabolizing enzyme mono-am[illegible] oxidase is inhibited by the presence of iproniaz[illegible] Stimulated uptake of 5 HT also seems improbal[illegible] since it was shown that in vitro irradiation [illegible] doses up to 100 Gy caused no alterations in [illegible] plasma values of 5 HT (VENINGA et coll) [illegible] creased permeability of the intestinal wall to [illegible] immediately after the irradiation can be exclud[illegible] since 5 HT was absent in the intestinal lumen b[illegible] before and after irradiation These negative findi[illegible] were not due to contamination with either $MgSO$[illegible] sucrose since both substances had no influence [illegible] the determination of 5 HT in control experime[illegible]

However in mice remarkably higher 5 HT va[illegible]

re found in the spleen immediately after abdomi irradiation with doses ranging from 5 5 to 7 5 Gy VARAGIĆ et coll (1963) also reported higher 5 HT les in the spleen of rats 24 h after 9 Gy γ irradia n They also observed a nearly 20 per cent fall in od platelets at that time but this was not suffi nt to explain the splenic rise in 5 HT GERSHON Ross (1966) showed that accumulation of 5 HT in e spleen occurred in cells of the reticulo-en thelial system The uptake of particles in this sys n was demonstrated to increase after irradiation LEMMING et coll 1970) Hence it might be possi to explain the present findings in the spleen by a mulated activity of the reticulo-endothelial cells a response to exposure to ionizing irradiation

The initially indicated suggestion of 5 HT as a chemical indicator for radiation injury seems not be of value in view of the irregular increase of HT in blood plasma of both animal species

SUMMARY

The radiation induced release of serotonin (5 HT) in bits and mice has a linear dose response relationship r a short range of approximately 0 75 Gy In both nal species a threshold value is present which is 1 Gy rabbits and 5 Gy for mice Doses higher than those g in the linear range give rise to lower plasma 5 HT es These lower values occur concurrent with in ased splenic 5 HT levels Stimulated 5 HT uptake of spleen may be responsible for the diminished increase plasma 5 HT

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INDUCTION OF PITUITARY TUMOURS BY COMBI... TION OF OESTROGENIC HORMONES AND ...Sr

A NILSSON P BIERKE I HARALDSSON and A BROOM... KARLSSON

...ously it has been shown (NILSSON & ...ACK 1973 NILSSON & BROOMÉ KARLSSON that oestrogenic hormones have a strongly ...ating effect on the cancerogenicity of ^{90}Sr in whereas corticosteroid hormones have the ...te action Thus when oestrogen was given to ...ice treated with ^{90}Sr in a dose of 29 6 kBq (0 8 body weight in spite of a striking acute mor ...ate the incidence of osteosarcomas increased ... twofold as compared with mice given ^{90}Sr ...4 ? versus 2 3 tumours per mouse respective ... a concomitant deduction of the tumour in ...n time with about 40 per cent (194±2 6 versus ...1 1 days respectively) This striking effect of ...e n did persist even if the ^{90}Sr dose was re... to 14 8 kBq/g body weight to female mice as ...ced by a tumour frequency of 4 1 versus 2 0 ...induction time of 251 0±4 1 versus 379 3±9 7 respectively On the other hand if methyl...solone was given for the whole surviving time second week to mice treated with 14 8 kBq body weight the tumour incidence was re... to 0 16 tumours/mouse as compared with 1 82 ...e given ^{90}Sr alone The mean tumour induc...me (589 days) as well as the mean survival ...(515±18 8) in the group given combined treat... was significantly prolonged as compared with ...r group (450±13 9 and 388±17 8 days)

...inally the present investigation was initiated ...er to analyse in more detail the promoting and ...ory effects of oestrogens and corticosteroids ...tively on the cancerogenicity of ^{90}Sr A prim...al was to find a ^{90}Sr dose low enough not to ... osteosarcomas but sufficiently high to produce bone tumours when combined with oestrogens On the other hand ... regards corticosteroid hormones the ideal objective was a ^{90}Sr dose which produced tumours at a level low enough to be completely inhibited by the combination of hormone and ^{90}Sr However during the elaboration of this material it was found that ^{90}Sr at the low levels used and in combination with oestrogen hormones as a side effect did induce pituitary tumours in an unexpectedly high frequency It was therefore decided to describe this material in a separate publication Unfortunately the present material has not been taken care of in such a way that immunohistochemical and fluorescence microscopy or electron microscopy could be applied Therefore the present report will mainly be concentrated upon an analysis of the frequency of pituitary tumours in the different groups used and their light microscopic characteristics

Materials and Methods

Three series (A B and C) of 75±3 days old CBA male mice were each given 3 different doses of ^{90}Sr (NO_3) intraperitoneally One of these series (A) was given ^{90}Sr only one (B) in addition with polyoestradiol phosphate (Estradurin Leo) subcutaneously at 3 consecutive times with intervals of one month The remaining series (C) was in addition to ^{90}Sr also given methylprednisolone (Depomedrone Upjohn) 1 mg subcutaneously every second week at 10 consecutive occasions A fourth series (D) of 70 animals was given only Estradurin The experimental condi...

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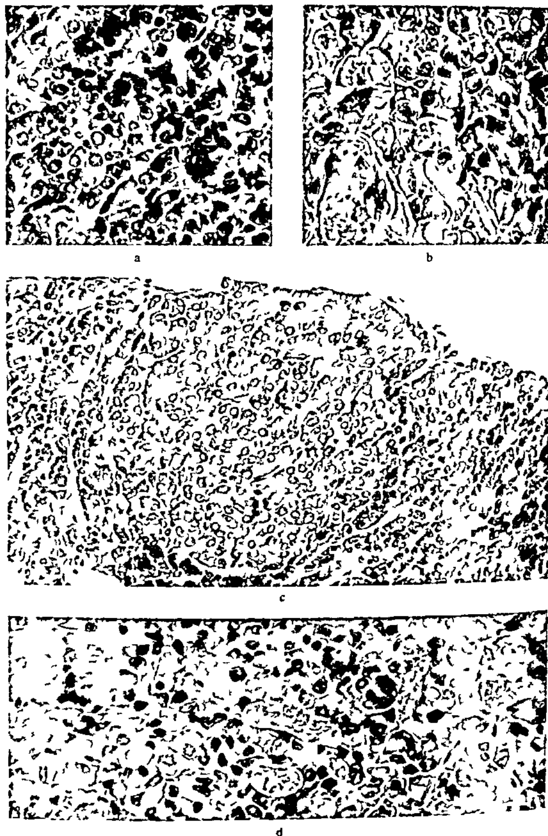


Fig 1 a) Normal pituitary gland pars distalis Acidophilic cells are orange yellow round or slightly ovoid with centrally placed nuclei Basophilic cells are purple slightly irregular with excentrically placed nuclei Tri PAS ×480 b) Pars distalis 45 days after treatment with oestrogen + ^{90}Sr (7 4 kBq/g b w) Strong acidophilic hyperplasia Slightly dilated sinusoids and accumulation of basophilic colloid Tri PAS × 480 c) P… distalis 540 days after treatment with oestrogen + ^{90}Sr (… kBq/g b w) Nodular acidophilic hyperplasia Tri PAS × … Pars distalis 77 days after treatment with oestrogen + ^{90}Sr (… kBq/g b w) Hyperaemia and sinusoidal dilatation Present… ance of basophilic and chromophobic cells Tri-PAS × 480

Table 1

al procedures CBA male mice treated with $^{90}Sr(NO_3)_2$ oestrogenic (Estradiol ... and ... steroid (Depomedrone) hor Sr was given at different dose levels (subgroups 1 2 and 3) and administered intr... 5±3 days of age (=day 0) The hormones were given repeatedly by subcutaneous injection

Sub-group	No of mice	Sr/g b w (kBq(μCi)) Day 0	Oestrogen (mg/mouse) Day −7	71	51	Corticosteroid (1 0 mg/mouse) Day −7	7	1	3		77	91	105	119
A 1	100	0 925 (0 025)	–	–	–	–	–		–		–	–	–	–
A 2	100	1 850 (0 050)	–	–	–	–	–	–				–	–	–
A 3	50	7 400 (0 200)	–	–	–	–	–	–			–		–	–
B 1	100	0 9 5 (0 0 5)	0 5	0 25	0 75	–	–	–	–		–	–	–	–
B	100	1 850 (0 050)	0 5	0 75	0 25	–	–				–	–	–	–
B 3	50	7 400 (0 200)	0 5	0 25	0 75	–	–	–			–	–	–	–
C 1	100	0 9 5 (0 025)	–	–	–	+	+				+	+	+	+
C	100	1 850 (0 050)	–	–	–	+	+				+	+	+	+
C 3	50	7 400 (0 200)	–	–	–	+	+			+	+	+	+	+
	0	–	1 0	0 5	0 75	–	–		–		–	–	–	–

nd the ^{90}Sr and hormone doses employed are d in Table 1 The mice (bred at the Swedish ch Institute of National Defence) were during ole experimental period kept in the same nd inspected twice daily They were grouped om and maintained in cages with 10 animals A commercial pelleted diet (Standard feed s and mice Astra Ewos) and water were d ad libitum

n reaching a moribund state the mice were by cervical dislocation and examined by dor tral radiography as a guide for locating s of the hard tissue The carcasses were y weighed as well as the liver spleen adren ds and kidneys These organs all macro hard and soft tissue lesions tumours and enologically suggested bone tumours were n Stieve s fluid The head was divided lon ally close to the midline and fixed with the n situ Hard tissues including the skull were fied in 20% formic acid Ordinary histologic ds were used and the sections were stained a matoxylin and eosin and with tri PAS ac g to Pearse

e the skulls were treated as described by fixa Stieve s solution and decalcification proce the material was as previously depicted un ately not suitable for immunohistochemical ds Therefore diagnosis of the pituitary rs could not be based on their hormone secre FURTH et coll 1976) but had to be classified according to their histologic features and affinity for stains On these grounds they were divided into chromophobic acidophilic and basophilic adenomas or in cases of malignancy as evidenced by infiltrative growth as carcinomas The tumours were sub divided in three groups according to the histologic appearances predominantly solid sinusoidal or trabecular structure

When comparing the tumour incidence between the different groups of mice the mean tumour induc tion rate (MTIR) was calculated It was defined as the regression coefficient of the tumour frequency in 100 mice as a function of time between T−SD and T+SD where T is the latency time between injec tion and the clinical appearance of the tumour and SD its standard deviation

Results

Early abnormalities An early histologic observa tion in mice treated with ^{90}Sr+ oestrogenic hormone (series B) was as compared with a normal material (Fig 1 a) a more or less conspicuous dilatation of the pituitary sinusoids with a strong accumulation of blood (Figs 1d 2) This initial congestion occurred in approximately the same frequency (15–25%) in all subgroups of series B but did appear somewhat ear lier (40 days) in the group given the highest dose of ^{90}Sr (B 3) than in the two other groups (90–115 days)

Another general feature of this series (B) was an

acidophilic usually diffuse hyperplasia in numerous glands long before the first appearance of tumours (Fig 1b c) In group B 3 this could be observed already about 50 days after the start of the experiment while in the other groups definite hyperplasia was evident first around day 250 to 275 A mere observation of the glands under low magnification easily revealed hyperplasia as a significant increase of the size of the pars anterior as compared with that of the normal gland cut in the same manner along the medial line (Figs 3 4)

Virtually in all cases the proliferating cells were acidophilic and generally somewhat larger than normally and were in the majority of glands diffusely distributed over the entire pars anterior (Fig 1b) In a few of these hyperplastic glands an additional finding was a strong accumulation of a basophilic homogeneous colloid in expanded sinusoids (Fig 1b d) Considerably less frequent than the diffuse acidophilic hyperplasia was a strictly focal or nodular acidophilic hyperplasia which usually appeared at multiple sites within the glandular parenchyma (Fig 1c)

In the ^{90}Sr groups (series A) hyperplastic glands were not frequent and were in contrast to the B series generally chromophobic (6) or consisted of both chromophobic and acidophilic cells (3) Only one case with predominantly acidophilic cells was observed In addition 2 cases of nodular hyperplasia were detected in the pars intermedia

In series C hyperaemia and sinusoidal dilatation was observed frequently Hyperplasias of both chromophobic and acidophilic type were found at several occasions In a few cases basophilic cells could also be found in an increased number as compared with normal glands In series D acidophilic hyperplasia was fairly often observed as well as hyperaemia and sinusoid dilatation

Survival time The survival times of the mice in the different groups are presented in Table 2 Generally the mice in series B survived a much shorter time than the mice of the corresponding dose groups of series A and C

Tumour incidence The incidence of tumour bearing mice in the different groups is given in Table 2 as well as the tumour induction time and the mean tumour induction rate (MTIR) It was obvious from the figures that the combination of ^{90}Sr in low doses and oestrogenic hormones had a strong syncancerogenic effect on the pituitary a fact which highly significantly potentiated the effect of either treat



Fig 2 Pituitary gland pars distalis 83 days after treatment w... oestrogen + ^{90}Sr (0 925 kBq/g b w) Extreme sinusoidal dilata... and hyperemia Tri PAS ×670

ment alone A comparison of the whole ^{90}Sr mate... (A) with that of ^{90}Sr + oestrogens (B) and w... oestrogen alone (D) revealed that the combi... treatment was more effective than ^{90}Sr or oestro... alone with almost a factor of 20 and 4 respect... ly It was also obvious that corticosteroids (C)... not have much influence upon the induction rat... pituitary tumours (Table 2)

A comparison of the cancerogenic effect in th... different groups of series B indicated as evalua... from the mean tumour induction rate in 100 m... (MTIR value) a clear difference between the gro... (B 1 versus B 2 $p<0.02$ and B 1 versus 1... $p<0.001$)

Tumour induction time The approximate late... time for tumour development (Table 2) was sho...

Fig 3 Normal pituitary gland 105 days old Haemat... eosin ×75

Fig 4 Pituitary gland 4 6 days after treatment with oestrog... ^{90}Sr (1 85 kBq/g b w) Hyperplastic enlargement of pars d... Tri PAS ×75

Fig 5 Pituitary gland 469 days after treatment with oestrog... ^{90}Sr (1 85 kBq/g b w) Sinusoidal adenoma Tri PAS ×60



Fig 3



Fig 4



Fig 5

Table 2

Incidence of pituitary tumours mean induction time and mean tumour induction rate (MTIR) in CBA male mice treated with ^{90}Sr (group A) ^{90}Sr + oestrogenic hormone (group B) ^{90}Sr + glucocorticosteroid hormone (group C) and oestrogenic hormone (group D) as coded in Table 1

Series	Sub-group	No of mice	Mean survival (days±SE)	Number of mice with tumour (%)	Mean induction time (days±SE)	MTIR (±SE)	t test B 2 and B 3 versus B 1
A	A 1	100	640±12	1 (1)	805		
	A 2	100	627±16	3 (3)	731		
	A 3	50	458±26	1 (2)	778		
B	B 1	100	446±25	44 (44)	641±14	0 166±0 007	
	B 2	100	500±18	37 (37)	629±15	0 144±0 008	p<0 02
	B 3	50	380±30	15 (30)	543±23	0 128±0 010	p<0 001
C	C 1	100	715±12	1 (1)	825		
	C 2	100	723±15	5 (5)	759		
	C 3	50	531±28	1 (2)	724		
D		70	502±21	7 (10)	670		

The mean tumour induction rate (MTIR) is defined as the regression coefficient of the tumour frequency in 100 mice as a function of time between T−SD and T+SD where T is the latency time between injection and the clinical appearance of the tumour and SD its standard deviation

in series B than in the other series Within this series it was also evident that the tumours did appear significantly earlier with increasing dose of ^{90}Sr Thus B 3 is clearly separated from both B 2 (p<0 01) and B 1 (p 0 001) However for B 1 and B 2 no difference was found

Tumour types The tumour material was classified according to its affinity to stains into predominantly acidophilic basophilic or chromophobic types

The tumours in series A and C all are chromophobic (Table 3) whereas in series B and D the tumours were either predominantly acidophilic or chromophobic In series B it is also notable that the majority of the tumours (65 9%) were acidophilic in group B 1—like in series D (60 0%)—whereas the reversed situation was found in group B 2 (43 8%) and B 3 (13 3%) From the histologic appearance of the material in series B and D the impression was gained that these tumours were preceded by an acidophilic hyperplasia which progressively could be transformed into initially acidophilic adenomas which during their progressive growth successively turned out to become chromophobic Therefore it was not always possible to classify the tumours as purely acidophilic or chromophobic since in most cases both types of cells were mixed

Thus the classification was based on the predominating cell type which means that a prepo[nderance] of chromophobic tumours could ha[ve] acidophilic cell elements or vice versa In ma[ny] occasions the chromophobic cells gave an impr[es]sion of being degranulated or very active evaluated from their large golgi complex whi[ch] sometimes contained a slightly pinkish or yellowi[sh] material The histologic characteristics of t[he] tumours could vary considerably within differe[nt] areas but based on the predomination of a cert[ain] structure 3 different types were generally discern[i]ble The most commonly observed type (Table Fig 5) was characterized by expanded sinuso[id] structures surrounded by large ovoid or cylindric cells with excentrically placed nuclei (Fig 6) T[he] lumen of the sinusoids was filled with erythrocyte[s] Not infrequently the sinusoids were heavily dilate[d] giving the tumours a cystic appearance particular[ly] when several sinusoids were fused by rupture their walls (haemorrhagic adenoma) In tumours these types occasionally follicular like structur[es] with indistinct or lacking lumen could be found Th[e] vast majority of the tumours were of the sinusoid[al] cystic type (Table 4)

The second most frequent type was the predom[i]nantly solid tumour which mainly consisted tightly packed small rounded or ovoid cells (Fig 7

Table 3

Incidence of pituitary tumours classified according to the predominant staining characteristics with tri PAS technique by Pearse. Treatment of the animal groups coded in Table 1

Series	Sub-group	No of mice	Tumours of pars distalis		Tumours of pars intermed.
			Acidophilic	Chromophobic	
A	A 1	100	–	1	
	A 2	100	–	3	
	A 3	50	–	1	–
B	B 1	100	29	15	–
	B 2	100	16	21	
	B 3	50	2	13	
C	C 1	100	–	1	–
	C 2	100	–	3	
	C 3	50	–	1	
D		70	5	2	–
Total		820	5[illegible]	61	

The third type was in longitudinal sections made up of long slender strands (trabeculae) of tightly packed cells with a prominent intervening stroma against which the peripheral cells of the strands were arranged in a pallisade like manner. On cross sections the tightly packed cells with indistinct cellular outline and rounded chromatin rich nuclei were forming rounded or irregular islands circumscribed by pallisading cells along the stroma. In the centre of these cell islands cells with somewhat excentrically placed nuclei could be found to form pseudofollicular structures consisting of a central blood vessel with pallisading cells. These tumours were in the present context said to have a trabecular structure (Fig. 8).

On several occasions adenomatous tissues were found to contain small isolated clones of cells which contrasted with the majority of the cells by

Table 4

Incidence of pituitary tumours of the pars distalis classified according to the predominant histologic type. Treatment of the animal groups coded in Table 1

Series	Sub-group	No of mice	No of adenomas			No of carcinomas		
			Sinusoidal	Trabecular	Solid	Sinusoidal	Trabecular	Solid
A	A 1	100	–	–	1	–	–	–
	A 2	100	–	–	3	–	–	–
	A 3	50	–	–	1	–	–	–
B	B 1	100	31	1	5	4	–	3
	B 2	100	29	1	2	4	1	–
	B 3	50	8	1	1	3	–	2
C	C 1	100	–	–	–	–	–	1
	C 2	100	1	–	1	1	–	–
	C 3	50	1	–	–	–	–	–
D		70	4	–	–	3	–	–
Total		820	[illegible]	[illegible]	14	15	1	6



Fig 6 Pituitary acidophilic adenoma 537 days after treatment with oestrogen + ^{90}Sr (0 925 kBq/g b w) Sinusoidal structure predominantly acidophilic Tri PAS ×235

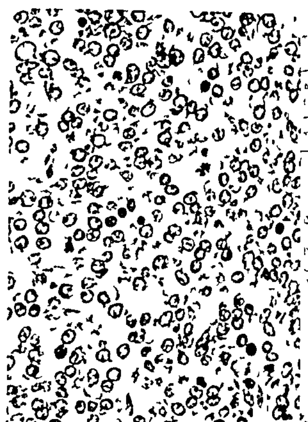


Fig 7 Pituitary chromophobic adenoma 617 days after treatm[illegible] with oestrogen + ^{90}Sr (1 85 kBq/g b w) Solid structure Tri [illegible] ×605



Fig 8 Pituitary chromophobic carcinoma 622 days after treatment with oestrogen + ^{90}Sr (1 85 kBq/g b w) Trabecular structure with cells pallisading along vascular stroma Tri PAS ×200

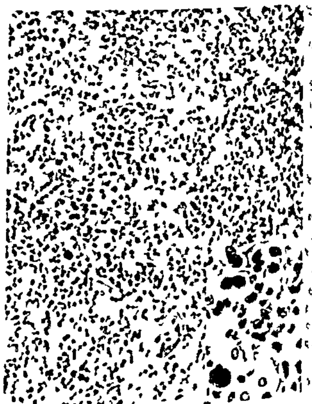


Fig 9 Acidophilic adenoma 41? days after treatment with oes[illegible] trogen + ^{90}Sr (1 85 kBq/g b w) Central part Island of [illegible] atypical predominantly chromophobic cells Tri-PAS [illegible] serted Magnification of central part Nuclear hyperchromas[illegible] pleomorphism and mitotic figure ×530

Table 5

me and number of pituitary tumours induced in groups of female CBA mice 75±5 days [illegible] The doses of 90Sr and oestrogen given

No of mice	Dose of oestrogen and day of administration: mg/mouse	Day	Dose of 90Sr (kBq/g b w)	Survival (mean±SE days)	No of tumours	Remark
50	–		14 8	388±17 8	[illegible]	
75	0 25	45 75	–	369±30 9	[illegible]	
50	0 25	45 75	14 8	253± 9 3		
50	0 25	75 105	14 8	89± 7 8	0	
50	0 25	105 135	14 8	377±17 3		N[illegible] (unpublished observation)
50	0 25	135 165	14 8	345±15 3	1 [illegible]	
45	0 25	255 285	–	660±38 5	1	
50	0 25	255 285	14 8	412±18 0		
50	–		–	873±22 7		
50	0 5	75 105 135	14 8	229± 9 4	[illegible]	Nilsson & Broomé Karlsson (1976)
143	1 0 0 5 0 25	75 105 135	29 6	147± 4 4	[illegible]	Nilsson & Rönnbäck (1973)

plasia chromatin rich and atypical nuclei (Fig Of a total of 96 tumours in series B 17 or 17 7 per cent were carcinomas as evidenced by pleomorphism atypical mitoses capsular penetration (Figs 11) and infiltration of the surrounding tissues A general impression was that the tumour cells during malignant transformation were loosing their acidophilic characteristics which seems to be mirrored by the fact that the majority (76 5% Table 4) of all carcinomas were chromophobic The histology of the tumours could vary considerably from field to field as well as cytologic features In some tumours multinucleated giant cells with large cytoplasmic invaginations of the nuclei were a frequent observation

In series A and C all detected tumours were chromophobic These tumours were generally in contrast to those induced by oestrogen or oestrogen and 90Sr with their conspicuous sinusoidal dilatation solid and composed of very large polygonal or ovoid cells with a large cytoplasm and usually a prominent intranuclear vacuole The nuclei were prominent clearly outlined and excentrically placed and mitoses were frequently observed In a few cases nuclear inclusions were discernible (Fig 12)

Of five tumours in group C 2 2 originated from the pars intermedia One of these was a microscopic adenoma composed of small tightly packed basophilic cells with hyperchromatic nuclei and atypical mitoses (Fig 13) The other was an adenoma of macroscopic extent and consisted of slightly pleomorphic basophilic cells with a high frequency of nuclear inclusions

Discussion

At an investigation originally performed for analysing the syncancerogenic effect of oestrogenic hormones and 90Sr on bone tumour induction unexpectedly a very high incidence of pituitary tumours was observed This was in contradiction to results obtained in several previous experiments in which 90Sr was given in combination with oestrogenic hormones However it is a wellknown fact that whole body irradiation local irradiation of the skull or treatment with oestrogenic hormones alone or in combination with irradiation may induce pituitary tumours (UPTON & FURTH 1953 GARDNER et coll 1953 CLIFTON 1959 KWA 1961 RUSSFIELD 1966 BERDJIS 1967) Oestrogenic hormones readily induce pituitary tumours in rats and mice whereas rabbits monkeys and guinea pigs are less susceptible (FURTH et coll 1973) The majority of such tumours seem to originate from acidophilic cells (CLIFTON & MEYER 1956 LUNDIN & SCHELIN 1962) and they are generally considered to be benign (FURTH et coll 1976) WAELBROECK VAN GAVER & POTVLIEGE (1969) thus have found that pituitaries about 9 months after cessation of oestrogen treatment can recover indicating that the early adenomatoid


Fig 10


Fig 11


Fig 12


Fig 13

...mours are oestrogen dependent Tumours induced ...rnal irradiation as well as by [131]At are usually ...philic (mammosomatotoropic YOKORO et coll ... 1964) In addition they are generally more ...ous than those induced by hormonal ...ement (FURTH et coll 1976)

With reference to the brief survey given the present findings are not surprising but have none the ...not previously been observed in experiments in which ^{90}Sr was combined with oestrogenic hormones From the results presented in Table 2 it is ...that the frequency of pituitary tumours in the different groups of series B treated by oestrogen is ...ely related to the dose of ^{90}Sr i e the incidence of tumours is decreasing with increasing dose of ^{90}Sr The reason for this is possibly associated with the fact that oestrogen renders the animals extremely sensitive to ^{90}Sr (NILSSON & RÖNNBACK ...) Therefore with increasing doses of ^{90}Sr the survival time of the animals will be too short for tumours to develop in the pituitary From the results presented in Tables 2 and 5 as regards tumour incidence and induction time there seems to be no doubt that ^{90}Sr strongly potentiates the effectiveness with which oestrogen induces tumours of the pituitary provided that the ^{90}Sr dose is low enough to allow a survival time of sufficient length for tumours to develop This also seems to be in agreement with the opinion (FURTH 1969) that the rationale of combination carcinogenesis is to bring about cell proliferation by some hormonal derangement and to hit the growing cells with mutagens which are most effective when the cells are in the DNA synthesis phase

In order to evaluate the relationship between Sr-dose and its influence upon the survival time and tumour frequency in oestrogen treated mice ...ous experiments (Table 5) were analysed in which higher doses ha... ...ployed than in the present series From th... ...us that increasing doses of ^{90}Sr have an ... deleterious effect on oestrogen treated ... evidenced by a high mortality and a red... survival time and this will explain ... tumours so rarely have been found in ...

Sinusoidal di... ...ment was found in all series but w... ...re conspicuous and in much earlier ... groups which were treated by oest... combination with ^{90}Sr The reason ... of these findings may be consid... reaction the degree of which may be ... to the stress of the treatment Another ... ing however occasional in this phase was the ... rence of a basophilic colloid material in the sinusoids Both these findings agree with observations that the vascular supply of mammotropic tumours is abnormal (FURTH et coll 1976) and that many of the enlarged sinusoids characteristic of these tumours contain colloid material and exhibit vascular stasis (TIBOLDI et coll 1968) This reaction was in the oestrogen group followed by or partly replaced by a diffuse or sometimes multifocal acidophilic hyperplasia of varying degree The hormonal secretion of these cells has not been tested However the general opinion is that acidophilic cells are associated with mammotropic and somatotropic function (SCHELIN et coll 1964) Furthermore most pituitary acidophilic tumours of the rat secrete both mammotropic and somatotropic hormones and have therefore been named mammosomatotropic tumours (FURTH et coll 1976)

The observations in series B and D seem to indicate that a diffuse or nodular acidophilic hyperplasia may be a primary step in a successive development of acidophilic adenomas many of which will later lose their tinctorial characteristics and turn chromophobic or transform into malignant tumours This development is largely in agreement with the observation that a fluid transition between hyperplasia conditioned and autonomous neoplasia exists (FURTH et coll 1976)

In contrast to series B and D cells which tinctorially were of chromophobic type predominated clearly in series A and C

Of a total of 115 tumours investigated only 2 were located in the pars intermedia whereas 52 acidophilic and 61 chromophobic were found in pars distalis It is notable that all the tumours in series A (5) and C

Fig 10 Pituitary acidophilic carcinoma 516 days after treatment with oestrogen + ^{90}Sr (1 85 kBq/g b w) Infiltration and destruction of basis cranii and growth within the bone marrow Very large golgi complex Tri PAS ×560

Fig 11 Pituitary chromophobic carcinoma 61. days after treatment with oestrogen + ^{90}Sr (0 925 kBq/g b w) Strong nuclear and cellular pleomorphism Penetration through and budlike growth outside the capsule Tri PAS ×560

Fig 12 Pituitary chromophobic carcinoma 778 days after treatment with ^{90}Sr (7 4 kBq/g b w) Chromophobic large cell tumour with solid structure Prominent golgi complex indistinct cell borders and atypical mitotic figures Tri PAS ×400

Fig 13 Tumour of pars intermedia 667 days after treatment with ... 1 85 kBq/g b w) and Depomedrone consisting of cells with indistinct cell borders and small round or ovoid hyperchromatic or heterochromatic nuclei Atypical mitoses Tri PAS ×315

(5) were chromophobic In the B series the yield of acidophilic tumours was high (65 9%) in the group given the lowest dose of ^{90}Sr whereas in the intermediate and highest dose groups these figures were considerably reduced (43 2 and 13 4% respectively) The reason for this may be related to factors such as a decreased hormone production or degranulation of the transformed cells The absence of acidophilia may also be related to a loss of information for specialized functions which is considered to take place during malignant transformation The fact that not less than 13 of 17 carcinomas (76 4%) in the B series were chromophobic as well as the observation that malignant progression in acidophilic adenomas generally was evidenced by islands of chromophobic cells with dysplasia and atypical cell nuclei of varying degree may be considered as a theoretical support for this view These observations are also in good agreement with previous findings that tumour cells in old acidophilic adenomas may lose their specific granules to such an extent that the acidophilic character may be overlooked (KRAUS 1953) and the fact that pituitary tumours which become malignant grow more or less chromophobic (FURTH et coll 1976)

Tumours of pars intermedia are extremely rare in the small laboratory animals and seems to have been described only in rats (RUSSFIELD) On the other hand spontaneous tumours originating here are the most common types of pituitary tumours in horses and the second most common type in dogs (CAPEN 1978) In horses females are more frequently affected than males (GRIBBLE 1972) Most tumours in dogs are endocrinologically inactive although there may be cases with an excessive ACTH secretion In horses a syndrome including muscular weakness hyperidrosis intermittent hyperpyrexia hirsuitism polydipsia etc develops mainly because the tumour exerts a pressure on the hypothalamic region (GRIBBLE) The two tumours detected in the present series were fairly small in size and have not demonstrated clinical symptoms

The tumours of pars distalis were divided into 3 histologic types The sinusoidal cystic tumours constituted an overwhelming majority which also seems to agree with the statement by WOLFF & WRIGHT (1947) that most of the smaller spontaneous adenomas are haemorrhagic and by FURTH et coll (1976) who found the vascular supply of primary mammotropic adenomas to be abnormal This also seems to be associated with the initial hyperemia and stasis of the sinusoids which c be observed long before cellular proliferation tumour transformation took place

SUMMARY

The present investigation was initiated to analys carcinogenic effect of combined treatment with ^{90}S oestrogenic hormones or corticosteroids in inbred mice Pituitary tumours appeared in a remarkably incidence in mice treated with oestrogens + ^{90}Sr i doses—0 925 kBq/g body weight (44%) and 1 850 body weight (37%)—as compared with mice treated ^{90}Sr only—1 and 3 per cent respectively The sy cerogenic effect is ascribed the oestrogen induced p eration of pituitary cells and their increased sensitiv radiation The reverse relation found between numb pituitary tumours and dose of ^{90}Sr in oestrogen tr mice is explained by the reduction of the survival with increasing dose Preneoplastic histologic chang the pituitary are described and pituitary tumours w mainly appeared in pars distalis are classified acco to the growth and tinctorial characteristics

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EFFECTS OF PROTON IRRADIATION OF THE L U ... ESCENCE ON INTRA AXONAL TRANSPORT OF ACETY ... NE AND CHOLINERGIC ENZYMES IN RAT SCIATIC ... RVE

S BOOJ A DAHLSTRÖM P A LARSSON K ROSANDER and B PERSENGREN

... sensitivity of peripheral nerves and ganglia to ... has been analysed in many animal experi... After irradiation with doses of 10 Gy and ... clear morphologic abnormalities have been ...ved in the peripheral nervous system (PNS) ...ER 1959 ANDRES et coll 1963 HOPEWELL & ...HT 1975) Functional and neurophysiologic ...s related to morphologic lesions have also ... observed (BERGSTRÖM 1962 CARSTEN & ...AN 1966)

Following postoperative irradiation of supra... ...ular glands in patients with carcinoma of the ...st functional disturbances as well as mor... ...logic abnormalities in the nervous system may ... Thus pareses and microscopically detectable ...s were described after treatment with roentgen ... of 5 MeV (STOLL & ANDREWS 1966) and sen... ...d motor disturbances were observed in pa... ... with breast carcinoma irradiated with ^{60}Co ...STLING et coll 1972) Lesions of the plexus ...alis were also noted in 17 per cent of such ...s by NOTTER et coll (1970) The neurologic ...cations appeared in these patients if the dose ... was 50 Gy or more over a period of 30 days ... latency period between the irradiation and the ...earance of the complications was between 5 ...s (STOLL & ANDREWS) and 20 months (NOT... ...et coll)

Several factors are of importance but the larger the total dose the shorter the total irradiation time and the fewer fractions the earlier do the symptoms and the morphologic lesions appear The late neurologic symptoms may be due to direct or indirect irradiation effects Direct irradiation trauma may for instance be due to a direct effect on the nervous tissue itself while indirect irradiation trauma may depend on disturbed nutrition of the nervous tissue caused by an impaired microcirculation

Direct irradiation effects on the nervous tissue proper (i e the neurons) are difficult to demonstrate since the effects on blood vessels and surrounding glia always interfere with the neuron itself One possibility is to observe the early effects of irradiation i e before the time when circulatory changes occur Glial reactions seem to occur within 24 hours after irradiation injury (cf HAYMAKER 1969) which means that this effect is presumably impossible to separate from pure neuronal injuries

One type of neuronal disturbance which might participate in the late neurologic symptoms is a changed intra axonal transport of various substances In both adrenergic and cholinergic (motor and autonomic) neurons substances which are essential for the maintenance of nerve transmission and nerve terminal integrity are synthesized in the perikarya and transported by an efficient intra

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axonal mechanism to the nerve terminals Also a retrograde transport of material from the periphery of a neuron towards its soma exists If the intraneuronal synthesis and transport of various substances (transmitter organelles metabolizing enzymes etc) were decreased because of direct irradiation effects on the soma motor disturbances (or defects of the autonomic sympathetic or parasympathetic nervous system) would occur after a latency period The intra axonal transport of acetylcholine (ACh) and the cholinergic enzymes cholineacetyltransferase (CAT) and ACh-esterase (AChE) in a motor neuron system of rat has been investigated following local irradiation of the motor perikarya in the lumbar intumescence The dose given was calculated in order to allow extrapolation of the results to clinical situations

Materials and Methods

Ninety ra of the Sprague Dawley strain (200–300 g both sexes) were used Under Nembutal anaesthesia and proper fixation the lumbar intumescence of the animals was irradiated with a single dose of a 187 MeV proton beam from the 230 cm synchrocyclotron at the Gustaf Werner Institute in Uppsala (LARSSON et coll 1959) The field size was 3 mm×8 mm and the absorbed single dose was 60 Gy The dose rate was about 15 Gy/min In one experiment in which the rats were used one day after irradiation 200 Gy were given The field was adjusted before irradiation by a combination of conventional radiography and autoradiography and was controlled after irradiation by the same procedures (Fig 1) Control animals were anaesthetized fixed and radiographs performed (short exposure) but not irradiated with the proton beam

One 7 or 30 days after the irradiation the animals were re anaesthetized with ether and the sciatic nerves bilaterally were crush-operated at mid thigh level (the unbranched segment of the nerve) as described by LUBINSKA (1959) A silk ligature (3×0) was carefully introduced under the nerve and a glass rod with a diameter of 5 to 6 mm was placed on top of and along the nerve The silk thread was then pulled firmly against the glass rod for 3 to 5 seconds This procedure caused a clearly defined straight crush of the nerve interrupting the axons but leaving the connective tissue sheaths intact One or two such crushes (with 1 mm distance between



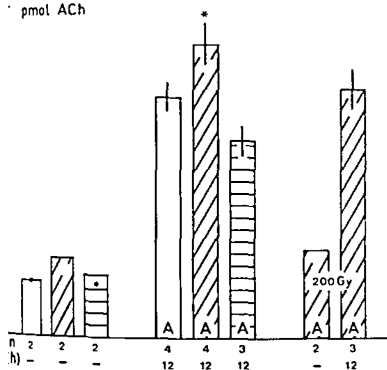
Fig 1 The irradiated part of the spinal cord of the rat is sented by the bright rectangle

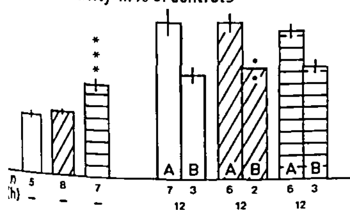


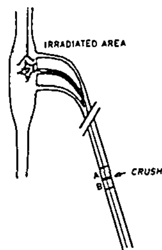
Fig 2 Longitudinal section from the ventral part of the lu intumescence of a rat irradiated 30 days earlier having the severe paralysis of the rats in the group Severe demyelinz to the right and fairly normal appearance of the white matt the left Demarcation zone at arrow Htx-eosin stain ×33

the 2 crushes) were made (Fig 3) Some rats in e group were used unoperated i e with intact sci nerves In the experiments on day 30 (Fig 4) nerves were crushed at 2 sites with 15 mm ap Twelve hours after the nerve crush operation rats were killed and 5 mm segments of the ne relative to the crush site were removed Bef dividing the nerves in segments the sciatic ner were carefully chilled on an ice cooled glass pl

Fig 3 The levels of ACh and the activities of AChE and CA the sciatic nerve of control rats (white bars) and rat irradia day (bars with oblique stripes) or 7 days (bars w h horiz stripes) before the final experiment In all cases 60 Gy was gi except in one ACh experiment where 200 Gy was given bars to the upper right) Individual observations (dots) means±SEM (vertical bars) are ind a ed n number of observa tions Some nerves were crush-operated 1 hours before de which is indicated below the bars crush (h) The location of sciatic nerve crush and of the various nerve segments (4 8 the sciatic nerve in relation to the irradiated area idered shown in the illustration to the right


pmol ACh
*
200 Gy
A
A
A
A
A
n 2 2 2 4 4 3 2 3
(h) – – – 12 12 12 – 12


AChE activity in % of controls
*
*
*
A B A B A B
n 5 8 7 7 3 6 2 6 3
(h) – – – 12 12 12


IRRADIATED AREA
CRUSH
A
B


CAT activity in % of controls
A B A B A B
n 32 22 20 20 12 10 8 12 9
(h) – – – 12 12 12

Fig 3

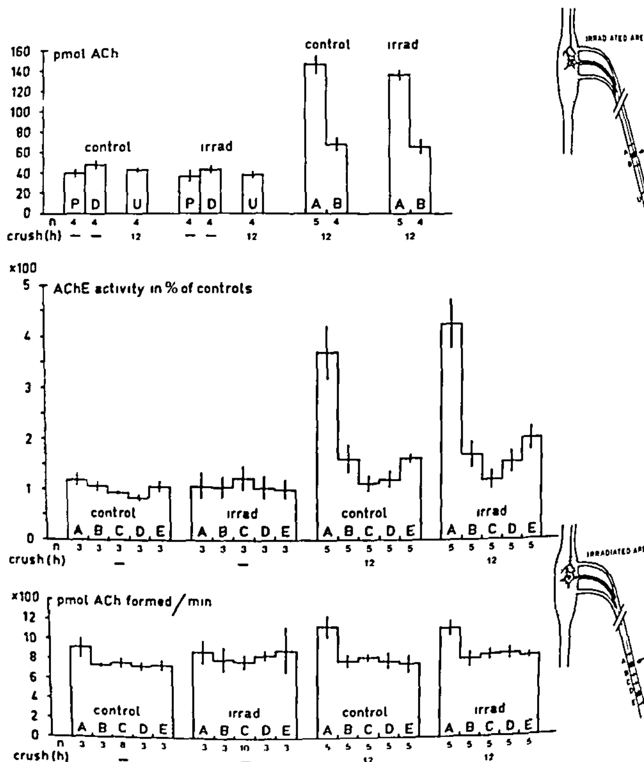


Fig 4 The levels of ACh and activities of AChE and CAT in sciatic nerves of control rats and of rats irradiated 30 days before the final experiment The values are given per 5 mm segment of nerve means ± SEM (vertical bars) are indicated Dose 60 Gy n = number of experiments Some nerves were crush-operated 12 hours before killing the rats crush (h) The location of the single crush (ACh) or double crushes (enzymes) in relation to the various segments (A–F and U) are indicated in the small illustrations to the right Letters P and D in the ACh bars for unoperated nerves indicate proximal (P) part of nerve corresponding to A and B and distal (D) part of nerve corresponding to D F and U in crush-operated nerves

Comparative segments were removed from the intact nerves (cf Figs 3 4)

For ACh assays 2 to 6 nerve segments were pooled The ACh was extracted in TCA as described by MACINTOSH & PERRY (1950) and assayed on the guinea pig ileum preparation (BLABER & CUTHBERT 1961) An antihistamine (mepyramine 2 5 x 10 ... was present in all solutions during the ACh ...

in the samples was identified by treatment of samples with purified AChE (Worthington s chemical New Jersey) and atropinization gut Both procedures inhibited the response the gut to samples The CAT activity was ured radiometrically according to GLOVER GREEN (197? experiment on day 1 and 7 Fig 3) according to TUČEK (1974) in the experiment on 30 (Fig 4) AChE activity in experiments on 1 and 7 (Fig 3) was assayed as described by ...M (1969) and in the experiments on day 30 ac ...g to TUČEK With the latter method the ...ity of both enzymes could be measured in the ... nerve segment homogenate

The results were analysed using Student s t test

The lumbar intumescence of some control rats ... of those in the 30-day group were removed ... in formalin embedded in paraffin sectioned at ... 10 μ and stained with a routine stain The ...ions were observed in a light microscope and ...ographed with Kodak TRI X Pan film (Fig 2)

Results

No indication of irradiation injury could be seen in ... on day 1 or 7 but on day 30 one of 12 animals ... a marked paresis of the hind limbs and 5 a mild ...eral paresis The remaining 6 of the 12 animals ... no evident symptoms The spinal cord of these animals was examined in a light microscope ... the white substance a varying degree of de...elinization was found in the irradiated zone the ... with the most severe symptoms having a marked ...myelinization (Fig 2) However the motor ...ia of the anterior horn appeared to be normal ... number and appearance The rats with mild ...sis had a moderate degree of demyelinization ... morphology was normal in the control rats

The ACh content of unoperated nerves appeared ... increased one day after irradiation with 60 or ... Gy When the irradiated nerves were compared ... all control nerves (Fig 3) the increase was ... significant (p<0 005) The accumulated ...nts of ACh proximal to the crushes after 12 ... were significantly higher than control in the ... given 60 Gy one day before the crush operation ... day 7 after irradiation the ACh-content in both ...ushed nerves and nerves crushed 12 hours ... was in the normal range (Fig 3) In the experi...t on day 30 no significant changes in ACh levels ... be observed when compared with the control group In the U segment the ACh content was 10 per cent lower than in the corresponding distal segment of uncrushed nerves in both the control group and the irradiated group (Fig 4) indicating that the transportable fraction of ACh was about 10 per cent in both groups (cf DAHLSTRÖM et coll 1974) No difference in ACh content or accumulation could be detected in the irradiated rats with no mild or severe neurologic symptoms

The AChE activity in uncrushed nerve was 4600±200 pmol of ACh split/min/5 mm (100±6%) in the control group (Fig 3) but increased to about 150 per cent on day 7 after irradiation (p<0 005) No changes were observed in the enzyme accumulations in nerves crushed 12 hours earlier In the experiment on day 30 the AChE activity of control uncrushed nerves was 4300±600 pmol of ACh split/min/5 mm nerve No clear gradient was evident along the nerve (A E segments) The distribution of enzyme activity in nerves crushed 12 hours earlier appears in Fig 4 (middle) In the A segment of control nerves proximal to the high crush about 370 per cent of enzyme activity was present On day 30 the AChE activity of both uncrushed and crushed nerves was not significantly changed from control levels

The CAT activity of uncrushed control nerves was 750±60 pmol of ACh formed/min/5 mm nerve and no gradient was found along the nerve (A–E segments Fig 4) In the A segment proximal to the high crush after 12 hours the amount of CAT activity was about 140 per cent of control No difference was observed 1 7 or 30 days after irradiation despite the condition of the irradiated rats

Discussion

The dose given to the spinal cord (60 Gy) was chosen to enable comparison with the clinical situation in which this dose with certainty would give functional disturbances Since the volume irradiated was rather small (approximate field size 8 mm × 3 mm) the dose was comparatively high because the biologic effects of irradiation are known to be related to both dose and volume (cf discussion in DE SAULT 1958)

The rat was chosen for the experiments for two reasons the intra axonal dynamics of rat motor neurons have previously been described (DAHLSTRÖM et coll 1974) and radiation induced symptoms similar to those in humans develop in rats

after irradiation of the spinal cord (INNES & CARSTEN 1961) Thus the rat is a suitable experimental animal for investigating radiation injury (VAN DER KOGEL 1977)

No neurologic signs of radiation injury could be observed in rats irradiated 1 or 7 days previously However on day 30 50 per cent of the rats had various degrees of paralysis of the hind limbs which could be related to the degree of demyelinization of the irradiated part of the spinal cord This observation is in accordance with HOPEWELL & WRIGHT who found that with an irradiation slit of 8 mm and a dose of 40 Gy an extensive demyelinization of the white matter and neurologic symptoms developed over a period of 1 to 2 months after irradiation The demyelinization was not associated with vascular injury but was limited to the irradiated region of the spinal cord only

The process of demyelinization had been described by several authors (CARSTEN & ZEMAN INNES & CARSTEN ZEMAN & SAMORAJSKI 1971) First it has been shown that this process does not necessarily precede tissue necrosis (ETSABLE PUIG et coll 1964 ZEMAN & SAMORAJSKI) Secondly the influence of the dose rate relationship is of vital importance for the appearance of demyelinization (McLAURIN et coll 1955) Thirdly it has been emphasized that radiation induced vascular disturbances are of major importance for demyelinization as a delayed effect (HAYMAKER) However this conclusion may not be valid for small fields signs of vascular injury having not been observed before the appearance of morphologic lesions in the gray and white matter proper (HOPEWELL & WRIGHT ZEMAN & SAMORAJSKI) In the present experiments demyelinization was observed already on day 30 while HOPEWELL & WRIGHT found demyelinization to develop between 1 and 2 months It is possible that the relatively high dose rate used in the present experiments may have hastened the onset of demyelinization

The hind limb paralysis which had developed after 30 days could not be related to changes in the intra axonal transport of ACh or cholinergic enzymes in the sciatic nerve In fact the only two significant changes observed after irradiation were (1) On day 1 after irradiation (60 Gy) both ACh content of intact nerves and ACh accumulation were higher than in the control group However in animals given 200 Gy no change in ACh accumulation occurred which at present seems to be unexplainable (2) On day 7 after 60 Gy the AChE activity of uncrushed n[...] was about 150 per cent of control On day 30 [...] irradiation no significant changes in axonal tran[...] were found neither in severely paralysed rats [...] in animals with mild symptoms Since the accum[...]tions of various substances proximal to a crus[...]eration reflect the perikaryal synthesis and [...] axonal transport of the enzyme or organelle in [...]tion the results clearly indicate that changes [...]tra axonal transport of ACh CAT or AChE ar[...] a major cause in the development of neuro[...] symptoms following irradiation

Previously brain areas were irradiated with higher doses (180 Gy CATRAVAS & MCHALE [...] which showed that cholinergic enzymes are [...]paratively resistant to irradiation In the presen[...]periments the irradiated cell bodies were [...] analysed for activities of CAT or AChE but [...] minor changes observed in the sciatic nerve a[...] well with these previous observations Howe[...] changes in proteolytic enzymes have been [...]monstrated by ZEMAN et coll (1960)

Recently it was demonstrated that transectio[...] the spinal cord at the thoracic level causes a r[...] and marked alteration in the intra axonal trans[...] of ACh and AChE in rat sciatic nerve (DAHLST[...] et coll 1978) In the present experiments a se[...] demyelinization of the white matter of the sp[...] cord did not influence the dynamics of choline[...] substances in the motor neurons in the sciatic ne[...] This may suggest that unmyelinated descen[...] fibre systems are more important than myelin[...] axons for regulating the intra neuronal dynamic[...] motor neurons

SUMMARY

The content and intra axonal transport of acetylcho[...] (ACh) and the cholinergic enzymes cholineace[...] transferase (CAT) and ACh-esterase (AChE) in sci[...] nerve were investigated in rats following single dose [...]ton irradiation of the lumbar intumescence of the sp[...] cord with 60 Gy or 200 Gy One 7 or 30 days a[...] irradiation nerve-crush operations were performed [...] hours before killing and the levels of ACh and enzy[...] activities in nerve segments relative to the crushes w[...] estimated by biologic (ACh) or chemical (enzy[...] methods The results indicate that alterations in in[...]neuronal dynamics of ACh and related enzymes are n[...] major cause for the development of neurologic sympt[...] of the motor system after irradiation and that descend[...] myelinated axons are of minor importance for the regu[...]tion of cholinergic substances in rat motor nerves

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IRRADIATION COMBINED WITH BLEOMYCIN TREATMENT OF SYNCHRONIZED CELLS IN CULTURE UNDER OXIC AND HYPOXIC CONDITIONS

J MIDANDER B LITTBRAND and F EDSMYR

...ent years many attempts have been made to ... combined modality treatment of tumors us... diation and Bleomycin (BLM) The clinical ...are as yet controversial and several reports ...tive (CACHIN et coll 1977) as well as positive ...L 1976 EDSMYR 1976 HANSEN et coll 1976 ... & KRISHNAMURTHI 1976) observations are ...e

...results obtained in laboratory experiments ...ombination of BLM and irradiation are also ...ersial particularly in regard to a possible ...tic effect of such a combined treatment on ...lethality The great variation in the sensitivi... ...fferent cell lines to the cytotoxic action of ...WENTYMAN & BLEEHEN 1973 SAKAMOTO ...nd the sensitivity variation even of the same ... to the drug under different experimental ...ns (TWENTYMAN & BLEEHEN TERASIMA ... 1975) may explain in part this contro... ...Another explanation may be the sensitivi... ...ion of the cells to BLM in different phases of ...cycle The present experiments were per... ...with the purpose to elucidate this question ...ly in regard to the early S and G phase the ...own to be particularly sensitive to both ir... ...l and BLM (TERASIMA & UMEZAWA 1970 ...CO & HUMPHREY 1971 SINCLAIR 1972 ...l et coll 1975)

Materials and Methods

Cells and synchronization procedure A substrain of the Chinese hamster cell line V79 was used propagated under standard tissue culture conditions The nutrient medium consisted of Eagle s medium in Earle s saline supplemented with 15 per cent fetal calf serum and antibiotics The cells in the radiation experiments were used after synchronization in their early S or G phase of the cycle

Synchronization was made by mitotic selection largely according to the method of TERASIMA & TOLMACH (1963) Cells in exponential growth phase were rinsed twice with trypsin buffer incubated for 15 min and thereafter shaken mechanically for 60 seconds The procedure was repeated once again and the cells were thereafter incubated for 45 min After a third final shaking the mitotic cells were collected again With the method the yield of mitotic cells was 1 5 to 2 per cent of the cell population at start

The specific phases of the cell cycle were determined in complementary experiments by pulse labelling of the selected cells with [12] IUdR diluted in trypsin buffer (Amersham specific activity 37–222 MBq/mg 1–6 mCi/mg) An aliquot of 0 1 ml IUdR solution (37 kBq 1 μCi) was added at one hour

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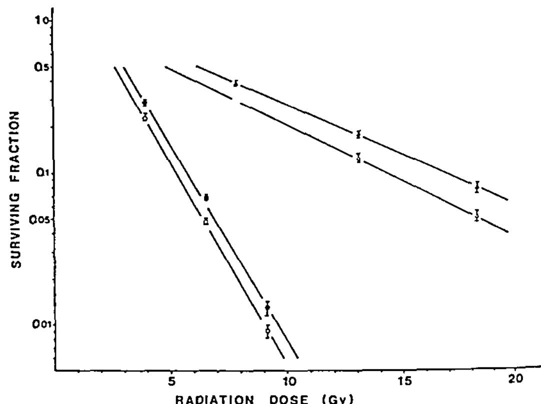


Fig 1 Radiation survival curves of Chinese hamster cells untreated or pretreated with Bleomycin The cells were in their early S phase when irradiated Irradiations were made under oxic (16 paired experiments) or hypoxic conditions in argon (14 paired experiments) Means are illustrated with SE The regression lines were calculated by least square analysis of the pooled data of the comparable experiments Oxic irradiation with Bleomycin (O) Oxic irradiation without Bleomycin (●) Hypoxic irradiation with Bleomycin (Δ) Hypoxic irradiation without Bleomycin

intervals during a 13 hour period to each of a series of triplicate Petri dishes (Ø 5 cm) containing the synchronized cells The labelled cells were incubated for 30 min and the activity was thereafter measured in the washed perchloric acid precipitate of the cells in a well type crystal scintillation counter The measurements of the relative activity indicated that the early S and G phase followed 3 and 9 hours respectively the explantation of the selected mitotic cells

Bleomycin treatment Bleomycin (H Lundbeck & Co Copenhagen) when added to the cells was dissolved in the culture medium in a final concentration of 60 μg/ml to which the cells were exposed for 2 hours As determined in a particular test with asynchronously growing cells 20±3 per cent of the cell population lost clongenic capacity due to the cytotoxicity of the substance under these conditions By pulse labelling with [125]IUdR as described the BLM treatment was found to delay progression of cells in the cycle for 15 to 30 min

Irradiation The synchronized cells were irradiated 3 or 9 hours after explantation in glass Petri dishes and incubated in the nutrient medium Immediately before irradiation the medium was drained to an extent that only 0 5 ml was le[ft to] cover the cells in order to facilitate gas exch[ange] The dishes with cover removed were placed [in a] plastic irradiation chamber flushed for 10 mi[n be]fore and during irradiation with either pure ox[ygen] or argon which contained oxygen in a concentr[ation] less than 1 ppm Carbon dioxide was suppleme[nted] to the gas flow to maintain pH 7 2 in the med[ium] When BLM was added the cells were expose[d to] the substance before and during irradiation f[or a] total of 2 hours After irradiation the cells [were] washed twice with trypsin buffer and reincubat[ed in] fresh medium Controls were sham irradiated u[nder] identical conditions as the irradiated cultures [The] reincubation time after irradiation was 8 days [with] one medium change on the second day The cl[ones] which developed were fixed and stained in [...] Thereafter the mean number of clones was e[stab]lished by counting 3 replicate dishes The fra[ction of] cells which survived irradiation was expresse[d as] the percentage of the unirradiated controls in [the] same experiment

Radiation was generated with a Siemens St[...]

Table 1

Numerical value of the parameters for the survival curves presented in Figs 1 and 2

Cell cycle phase	No of paired replicate experiments	BLM conc (μg/ml)	Plating efficiency (per cent)	Slope constant of survival regression Mean ± SE (log units/Gy)	D Mean (Gy)	Extrapolation number	
						Mean ± SE (log units/Gy)	Arithmetic value of mean
Early S	16	80	38±3	−0 6 0±0 019	1 61	0 96±0 10	2 61
		–	5?±4	−0 600±0 023	1 67	1 1 ±0 13	3 07
G	_6	80	27±3	−0 580±0 014	1 72	1 10±0 06	3 01
		–	46±6	−0 580±0 018	1 72	1 35±0 06	3 85
Early S	14	80	49±4	−0 169±0 009	5 9?	0 11±0 11	1 1
		–	64±5	−0 151±0 007	6 6?	0 _4±0 07	1 ?7
G	10	80	33±4	−0 166±0 01	6 02	0 ?4±0 14	1 27
		–	52±7	−0 152±0 01?	6 58	0 16±0 14	1 17

ulated by least square analysis of the survival data in each experiment separately
iprocal of the mean regression coefficient
rsection of the regression line with the ordinate at zero Gy

Table 2

e of extrapolation number by Bleomycin treatment for al curves of the oxically irradiated cells in the experiments presented in Table 1

Decrease of extrapolation number			
Mean ± SE (log units/Gy)	Arithmetic value of mean	t	p
−0 1_5±0 061	0 88	2 05	<0 05
−0 189±0 063	0 83	? 98	<0 0?

Table 3

Increase of regression coefficient by Bleomycin treatment for the survival curves of the hypoxically irradiated cells in the experiments presented in Table 1

Cell cycle phase	Increase of regression coefficient			
	Mean ± SE (log units/Gy)	Arithmetic value of mean	t	p
Early S	0 03?±0 014	1 03	? 9	<0 05
G	0 013±0 009	1 01	1 4_	>0 1

oentgen Unit at 220 kV and 15 mA The half layer was 0 3 mm Cu and the dose rate 3 8 n at the bottom of the culture dishes FD=40 he dose delivered was measured by a Philips iting dosimeter

Results

ne series of experiments the effect of BLM ent of the radiation sensitivity of Chinese er cells in their early S phase was determined irradiation under oxic (16 replicate experi) and hypoxic (14 replicate experiments) con s The survival was determined in all cases exposure of the cells to 3 different radiation which reduced survival to between 40 and 1 per cent Fig 1 illustrates the survival curves calculated by statistical exponential curve fit from the pooled data according to the single hit multi target model The curves show that when irradiation was performed under oxic conditions BLM treatment reduced the extrapolation number n and had only a minor effect on D_0 This can be considered as indicating that the substance interferes with the recovery of the cells from sublethal damage On the other hand when the irradiation was performed under hypoxic conditions BLM treatment increased the slope of the survival curves i e decreased D_0 and had only a slight effect on n The numerical value of the survival curve parameters are given in Table 1 Statistical analysis (Tables 2 and 3) indicated that the decrease of n under oxic conditions and the

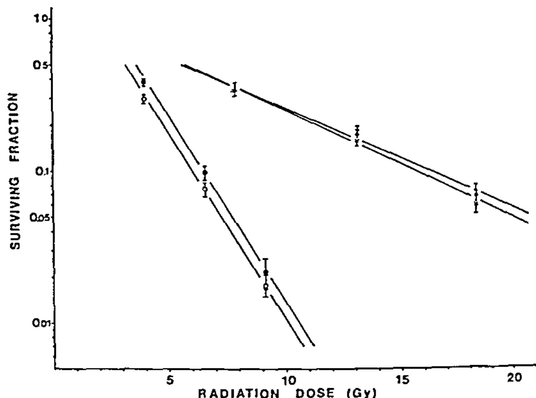


Fig Radiat ... for cells in G phase untreated or pretreated ... Means ± SE are presented from 26 paired oxic and 10 paired hypoxic experiments Symbols a Fig 1

increase ... slope of the survival curves under hypoxic conditions are significant

In ... series of experiments the effect of BLM treatment was tested upon G phase cells As illustrated in Fig 2 BLM treatment (26 replicate experiments) again decreased the extrapolation number of the cells irradiated under oxic conditions without any effect on D In contrast the parameters of the survival curves of the cells irradiated under hypoxic conditions (10 replicate experiments) are not affected by the substance to any significant degree As indicated in Table 2 the decrease of the extrapolation number of the cells under oxic conditions is again statistically significant

Discussion

Cell lines of different origin show a great variation in sensitivity to BLM at different concentrations (TWENTYMAN & BLEEHEN SAKAMOTO) The cell line used in the present experiments appears to be particularly resistant to BLM While BLM already at 2 μg/ml concentration killed 20 per cent of a HeLa cell population (TWENTYMAN & BLEEHEN) a comparable effect on the Chinese hamster cell line growing asynchronously was found first at a concentration of 80 μg/ml As can be calculated from t plating efficiencies indicated in Table 1 the se sitivity of early S phase cells to the cytotoxic effe of BLM is slightly higher (23 and 27 per cent kill under hypoxic and oxic condition respectivel while the sensitivity of G cells is considerably hig er (37 and 41 per cent respectively) than the se sitivity of asynchronously growing cells

The observation that the extrapolation number of the oxic survival curves of the G and early phase cells decreased when irradiation was co bined with BLM treatment while n of the hypo survival curves remained unchanged indicates specific sensitization of the oxically irradiat cells to radiation by interference of BLM with t recovery process form sublethal radiation dama The data indicate furthermore that recove is inhibited to a greater extent in the case of G than early S phase cells A greater sensitivity G phase cells to the cytotoxic effect of BLM trea ment is also suggested by the lowered plating eff ciency of the cells after treatment with the su stance and is in agreement with other reports on t specific sensitivity of the cells in this particul phase of the cycle (TERASIMA & UMEZAWA BAR RANCO & HUMPHREY) Interference of BLM with

ation recovery process has been observed previ ly when asynchronously growing cells were lied under oxic (MATSUZAWA et coll 1972) as l as hypoxic (SHRIEVE & HARRIS 1979) condi s It can be calculated from the present survival ves that the enhancement factor for the sensitivi f early S cells irradiated under oxic conditions is 3 and 1 07 and of G cells 1 17 and 1 06 at the 50 l per cent survival level respectively The in iction between radiation and BLM thus manifests lf most distinctly when relatively small radiation es are given Based on this conclusion radiation apy combined with low doses of Bleomycin has n used in the treatment of malignant tumors in mouth in a preliminary clinical trial The prelimi y observations (LINDHOLM et coll to be lished) agree with the experimental findings

he D_0 of the radiation survival curves was not d to be changed by BLM treatment with the eption of the hypoxically irradiated early S cells his case a statistically significant sensitization is arent by a dose modifying factor of about 1 1 s value is approximately the same as the one for cells irradiated under oxic conditions due to the airment of the recovery from sublethal damage he low dose range when they may cancel each r

i view of the differences in the reaction of G_2 and y S cells the overall result of a combined BLM tment and irradiation of the heterogeneous asyn nous tumor cell population which grows in vivo depend among other factors upon the particu proportion of oxygenated and hypoxic cells in erent phases of the cycle However whatever proportions any superiority of the combined itment in obtaining improved therapeutic results l depend upon a specific affinity of BLM to the plastic tissue Such a specificity has been ob ved in some cases (CAPUTO 1976) though it may t be a general phenomenon The sensitization of ll oxygenated normal tissues may require a de ease of the therapeutic radiation doses in all ses When tumor specific affinity exists the en nced sensitivity of the well oxygenated cells in the nor may be of a particular advantage in the com ned treatment These cells may be killed preferen ally leading to an enhanced reoxygenation of the lls in the hypoxic compartment Information on e tumor specific affinity of BLM in the individual ses seems to be essential for the design of treat ent with the drug in combined modality

SUMMARY

Bleomycin treated cells are sensitized to radiation de livered under oxic conditions both in the early S and G_2 phases of the cycle irradiated under hypoxic conditions sensitization occurs only in the early S phase This differ ence in the sensitizing effect of the drug is discussed in regard to the possible clinical advantages of a combined treatment of tumors with irradiation and Bleomycin

ACKNOWLEDGEMENTS

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NUCLEAR IMAGING OF PULMONARY METASTASES IN THYROID CARCINOMA

A M WORM I HOLTEN and E TAANING

Well differentiated thyroid carcinomas (papillary follicular and mixed types) usually have a prolonged course though the incidence of metastases is high Metastases to the cervical lymph nodes occur in 35 to 80 per cent and distant metastases usually to the lung in 10 to 40 per cent of the patients (WOOLNER & coll 1961 CRILE 1971 LINDAHL 1975 RASMUSSON 1978) The distribution of metastases differs in the follicular and the papillary carcinomas lymph node metastases being most common in the papillary carcinomas whereas distant metastases occur most frequently in the follicular carcinomas

It has been repeatedly documented that chest radiography does not detect all pulmonary metastases but that scintigraphy with ^{131}I may demonstrate some that are not radiographically detectable (CATZ & STARR 1956 ERNST & HEINE 1961 BARRETT & STENBERG 1965 BONTE & MCCONNELL 1973) Therefore ^{131}I whole body scintigraphy was compared with chest films in a series of patients with well differentiated thyroid carcinoma in order to obtain an increased knowledge of the value of these methods

Material and Methods

The patients were referred for evaluation and treatment at this oncologic centre ^{131}I treatment was given to patients with supposed or confirmed tumor tissue outside the thyroid gland

During a 3 year period (1976 to 1979) 161 whole body scans were performed in 52 patients (17 males and 35 females mean age 50 years range 14–84 years) with well differentiated thyroid carcinoma diagnosed between 1960 and 1978 but without evidence of co existing malignant disease

All whole body scans from these 52 patients were reviewed with special reference to abnormal pulmonary ^{131}I accumulation and compared with routine chest films

Due to varying surgical practice or degree of operability in 20 patients total thyroidectomy was not carried out

The ^{131}I whole body scintigraphy was performed 2 days after oral Na ^{131}I intake using a gamma camera with a whole body imaging accessory (Nuclear Chicago Pho/Gamma III HP IV and a 410 keV parallel collimator) The ^{131}I doses varied from 0 74 to 4 625 GBq (20 to 125 mCi)

Thyroid hormone and dietary iodine intake was avoided for 1 to 2 weeks before the ^{131}I administration A hypothyroid state was confirmed by laboratory tests including thyroid stimulating hormone (TSH) determination before scanning

Results

Pulmonary metastases were evident in 14 of the 52 patients diagnosed at the same time as the primary thyroid carcinoma in 7 patients In 7 patients the

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lung metastases were detected 5 to 15 years (mean 10 years) after the diagnosis of the thyroid carcinoma

The lung metastases were demonstrated at scintigraphy as well as at radiography in 7 patients Four patients had lung metastases demonstrated only at radiography all with subtotal thyroidectomy

At whole body scintigraphy 3 patients had abnormal pulmonary ^{131}I uptake though no abnormality was found at repeat chest radiography within the same period

Discussion

Some of the decisive factors for the survival of patients with well differentiated thyroid carcinoma are age histology and the presence of distant metastases (FRANSSILA 1975 MAZZAFERRI et coll 1977 BEIERWALTES 1978) Surgery followed by ^{131}I treatment has reduced the mortality rate (VARMA et coll 1970 HARNESS et coll 1974 LINDAHL BEIERWALTES)

As for lung metastases a recent Danish survey of thyroid carcinoma reported a frequency of radiographically demonstrated lung metastases in the well differentiated carcinomas of less than 10 per cent at autopsy of 50 cases pulmonary metastases were revealed in 18 8 of these with no abnormality detected on chest films during life (RASMUSSON)

In the present series lung metastases were evident in a total of 14 of 52 patients at ^{131}I whole body scintigraphy or on chest films The patient material is selected insofar as the ^{131}I whole body scintigraphy was carried out only in patients with extrathyroidal tumor tissue Therefore the number of patients with lung metastases in this series does not reflect the all over frequency of lung metastases in well differentiated thyroid carcinoma Seven of the 14 patients had lung metastases demonstrated both at radiography and at scintigraphy

The value of ^{131}I whole body scintigraphy in addition to radiography is demonstrated in the 3 patients with an abnormal scan but negative radiography These cases are in accord with those of other reports both with respect to the combination of abnormal ^{131}I scintigraphy and normal chest films and to the efficacy of treatment as indicated by the loss of capability of the metastases to accumulate ^{131}I (CATZ & STARR ERNST & HEINE BARRETT & STENBERG TURNER & WEIR 1972 BONTE & MCCONNELL)



Follicular thyroid carcinoma in a 74 year-old woman After s[urgery] treated with ^{131}I a) Whole body scintigraphy after f[irst] dose Diffuse and nodular uptake in the lungs b) After last d[ose] (total dose 13 875 GBq 375 mCi within 6 months) normal sc[intigraphy] No abnormality detected on repeat chest films

Four patients had metastases demonstrated on[ly] at chest radiography Failure of the tumor to co[n]centrate iodine due to low TSH stimulation could [be] ruled out as the cause of negative scintigraphy sin[ce] these patients had laboratory values indicating [a] hypothyroid state with a high endogenic serum co[n]centration of TSH Subtotal thyroidectomy was pe[r]formed in these patients and the initial ^{131}I uptake [in] the thyroid remnant was of such a degree as to a[c]count for the lack of a demonstrable amount [of] iodine outside the neck (BEIERWALTES) Two [of] these patients died before further treatment could [be] instituted

In the third patient it was not possible to ablat[e] the thyroid remnant despite treatment with ^{131}I to [a] total of 27 75 GBq (750 mCi) This patient died wit[h] widespread metastatic disease

Although the thyroid remnant was ablated aft[er] several ^{131}I treatment doses in the fourth patien[t] the radiologically evident metastases remained no[t] demonstrable by nuclear imaging up to the death [of] the patient 1 1/2 years after the diagnosis wa[s] established Autopsy confirmed the diagnosis o[f]

...d carcinoma with pulmonary metastases The ...n why the pulmonary metastases remained ...demonstrable at scintigraphy in this patient can ...be speculated upon

...lure to accumulate 131I in distant metastases ...ugh the thyroid gland is totally ablated has ...described by HARNESS et coll and CHARBORD ...ll (1977) Furthermore PREISMAN & HALPERN ...8) reported that low tumor versus background ...ratio may camouflage 131I uptake in small pul... ...ary metastases

...e figure of 14 patients with lung metastases in ...present series can only be considered as a mini... incidence The result clearly demonstrates that ...er 131I whole body scintigraphy nor chest radio... ...s alone will reveal all cases of lung metastases ...s type of tumor

SUMMARY

...onary metastases were diagnosed in 14 of 52 pa... ...with well differentiated thyroid carcinoma by ...hole body scintigraphy or radiography Three pa... had metastases only demonstrable on scintigraphy ...as chest radiography was diagnostic in 4 patients ...ormal scintigraphic findings Thus in this type of ...d carcinoma neither 131I scintigraphy nor routine ...films alone will reveal all cases of pulmonary meta...

...uest for reprints Dr A M Worm Department of ...l Physiology The Finsen Institute Strandboule... ...49 DK 2100 Copenhagen Denmark

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MICROPROCESSOR SYSTEM FOR TRACKING ISODENSITY LINES IN FILM DOSIMETRY

R VAN DER LAARSE and J DE GANS

The recent fall in price of microprocessors offers resting possibilities for new approaches in plot isodensity curves for radiation dosimetry A ve inexpensive equipment is now described ich uses an INTEL 8080 microprocessor The ratus tracks the isodensity line directly by de g the step length and the step direction from eding density measurements

he tracking procedure is based on the following nts

) The length and the direction of each new step based on the combination of up to the last 10 ious steps After each step the local density is sured and a new step length and direction is puted

) Isodensity tracking is initiated with a preset ting position taking the local value as isodensity e Also the direction for the first few steps must tered if default values are unsuitable

) The step length is adapted to the local density ient Density gradients in the central region of beam are considered as the normal ones At ll depths high dose variations exist at the beam s this will produce high density gradients on a Inside the beam at greater depths and in re s outside the direct beam the corresponding sity gradient is low For steps not coinciding the mean direction the procedure checks ther the local gradient belongs to the high nor or low gradient category

) The ragged appearance of isodensity lines sed by small film irregularities dust particles etc is reduced by defining an upper limit between the local signal and the preset isodensity value only outside this limit the spot will start to diverge from the mean direction

(5) The tracking procedure copes with the appearance of film stains (caused by dust particles for instance) of a diameter greater than the step length If the spot enters a stain it tends to move in a circle inside the stain because it has lost its orientation to the isodensity line The direction of the last steps are retained to discern this circling

Equipment

The equipment consists of a densitometer a moving film table coupled with a drawing board a fixed pen and a microprocessor (Fig 1)

Any densitometer can be used which fulfils the following requirements The light spot on the film should have a diameter of less than 1 mm The resolution of the densitometer should be about 0 1 per cent of the full scale with density 1 0 i e the lowest density which should give full scale response with maximum amplification The densitometer signal is fed by means of an AD converter (11 bits) into the microprocessor

Two stepping motors move the film drawing board assembly in two orthogonal directions Combining both motors a step can only be in one of the 8 principal directions (A Fig 2) When the equipment

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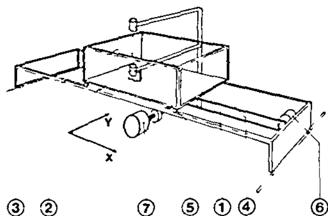
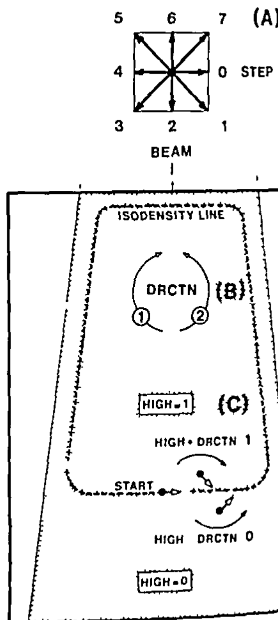


Fig 1



Fig

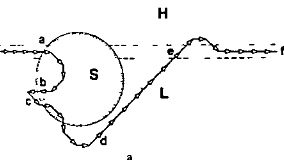
Fig 1 I ... Photomultiplier (2) Lamph ... (4) Drawing board (5) Drawing pen (6) Steppi ... (7) Stepping motor with high precision thread d ...

Fig ... king procedure (A) The eight directions for a step ... ing the drawing board in two orthogonal dir ... of the measuring light spot is either ... lockwise ① indicated by DRCTN=1 and DRCTN ... tively (C) The inner area of the isodensity ... indicated by HIGH = 1 the outer area by HIGH ... to start the isodensity tracking (START) ... The default start direction is LR = 0 The ... steps are governed by HIGH + DRCTN

is switched on high precision potentiometers indicate to the processor the table assembly position All further positioning is made by counting the impulses given to the stepping motors The drawing board moves under a rigidly fixed drawing pen the lift mechanism of the pen is controlled by the microprocessor software A similar mechanical coupling for drawing isodose curves is given by HASKARD (1975)

The microprocessor of the isodensity tracking equipment consists of an INTEL 8080 processor 6 k bytes Read Only Memory for the software instructions including the tracking procedure and 1 k bytes Random Access Memory for data handling and program stack

A numerical keyboard is available on th panel to manipulate the film t assembly or to enter a series of the film In the manual mode the table assembly and the corres ty are displayed by 4 digits ual mode is used to meas the film and to adjust the ingly Next the (x y) co film are entered from wh to start These position percentage depth dose d metric methods This ap the problem of convert dose

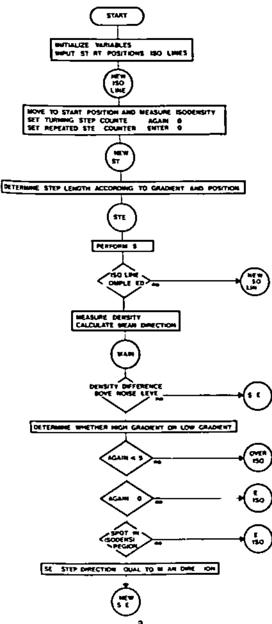


a

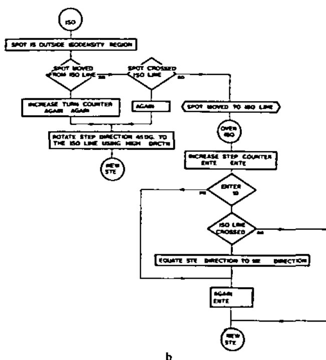


b

3 a) Movement of measuring spot with respect to a stain region along isodensity line inside which steps are taken in the mean direction H Region with densities higher than in region I Region with densitities lower than in region I S Region with high density a Spot enters high density stain 4 times turning and spot moves in reverse direction (AGAIN = 5) b Step in reverse direction is repeated and the number of these steps stored in ENTER. c Spot leaves stain In this example the isodensity value is crossed also and the step direction is equated to the mean direction However the spot moves away from the isodensity line and the turning process restarts d After a few turns the spot moves towards the isodensity line and steps are repeated e Spot in isodensity region Next step in mean direction moves spot out of the isodensity region f Spot in isodensity region Mean step direction has taken the correct value b) Actual tracking around a circular stain



a



b

Fig 4 Flowchart of the tracking procedure

Tracking procedure

The procedure starts with moving the measuring light spot (called spot hereafter) to a position entered manually the corresponding density is taken as the isodensity value The pen is lowered and the first few steps are done in either the default direction (LR=0 A Fig 2) or in a direction entered manually

The procedure uses three preset density difference levels The smallest one prevents the procedure to act on small differences due to noise between subsequent signal values A second density difference level determines a zone around the isodensity value If the measured density lies in this zone the next step will be a step in the mean direction This zone around the isodensity line will be called the isodensity region

Within the isodensity region a step is always a 1 mm step in the mean direction Every time a step outside the isodensity region is taken in a direction not coinciding with the mean direction the measured density i compared with the previous one If this local den difference is smaller than the third preset densit ifference level the next step will be a 1 mm step If greater a step not in the mean direction wil 0 5 mm step This step is in the high gradi r

Let DF 0 ndicate anticlockwise direction and DRC I the clockwise one (B Fig 2) Movem measuring light spot into the inner are set able HIGH to 1 when entering the outer are HIGH is set to 0 When a step moved the spot aw is from its isodensity value the necessary change in direction for the next step is given by HIGH + DRCTN (C Fig 2)

If a previous step moved the spot correctly the next one is a step in the mean direction of the last 10 steps rounded to the nearest 45

Outside the isodensity region the next step direction will be rotated 45 towards the isodensity line If the spot then moves towards the isodensity value this step will be repeated until the isodensity value is reached or passed However if the spot moves parallel to or diverges from the isodensity value the next step direction will be again rotated 45 to the previous one (Fig 3)

After 4 times repeating this turning the step direction has reversed and the measuring spot tends to move in circles The variable AGAIN counts the number of consecutively increasing or decreasing step directions after 4 times AGAIN equals 5 (Fig

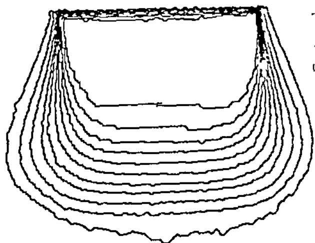


Fig 5 Isodensity curves at 20 MeV electron radiation (field 10 cm × 10 cm) drawn by the tracking procedure The isodensity tracking was started on the central axis at the positions of the 90 80 10 5 and 3 per cent depth dose Note the following characteristics The beginning and the end of a tracked curve do not necessarily coincide cf the 90 per cent line due to introduction of an isodensity region along the isodensity line The 3 per cent line shows some meandering in the low gradient region inside the beam

3) Then all following steps are taken in the last direction However if after 10 steps the spot still moves away from the isodensity line the 45 turning process restarts

The completion of the isocurve is detected by testing after each step whether or not the spot has entered a small region around the starting position

More details can be found in the flowchart of the tracking procedure (Fig 4)

Results and Discussion

The tracking procedure has two advantages a compared with other techniques The tracking speed is maximal and a direct coupling with a drawi board is possible because all steps have a small c zero divergence from the isodensity line An X Y pe recorder is thus not needed which reduces the cost of the whole equipment substantially

The combination of global movement and mea direction gives the procedure a remarkable capacit to handle irregularities like film stains or isodensit lines starting from a film edge at the entrance or e side of the beam Meandering of the isodensity li occurs only in regions with very low density gra dients (Fig 5)

A single set of values for the different densit difference levels and the number of last steps t retain for the mean direction suffices for all densi

.. tested Since the density differences are all ... e to the isodensity value the accuracy of the ...sity curve obtained is to a high extent inde...nt of the gain of the densitometer provided ...ter is sufficiently high

SUMMARY

...g a microprocessor a method for plotting isodensity ... film dosimetry is described The procedure moves ...asuring spot directly along an isodensity line using ...ation obtained in preceding steps to determine the ...ep For registration of the curves no X Y pen recorder is needed The essentials of the procedure together with a flowchart are given

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PHOTON ENERGY SPECTRA OF A HEAVILY FILTERED 300 kV THERAPY UNIT

T S CHEN K R KASE and B E BJARNGARD

Knowledge of the energy distribution of roentgen beams used in radiation therapy is a useful aid in the design of beam modifying filters for the machine such as flattening and hardening filters as well as wedges and compensators It is also valuable for detailed analysis of absorbed doses in the patient e g in determination of the f factor for bone During the past decade several reports on measurements of spectra for high energy roentgen machines have appeared (BENTLEY et coll 1967 JESSEN 1973 LEVY et coll 1974) However before the recent report on spectral measurements on orthovoltage machines up to 300 kV (SEELENTAG & PANZER 1979) only one seems to have been published (GOODWIN 1966) since bremsstrahlung spectra in this energy range were presented more than 20 years ago (HETTINGER & STARFELT 1958) This sparsity of data no doubt reflects the reduced utilization of orthovoltage roentgen rays in radiation therapy

The Philips RT 305 machine is designed to produce photon spectra such that the f factor in bone is low thus allowing irradiation without excessively high doses to bone structures within the irradiated volume (WICHMAN 1965 RADEMACHER & NAGELE 1975) This is achieved by the use of 300 kV acceleration potential and heavy filtration The intended use is for irradiation at shallow depths and a rapid fall off is assured by the use of short source to-surface distances (SSD) The treatment cones available have individual filters and produce beam qualities ranging from 6 1 mm Cu HVL at 6 cm SSD to 3 2 mm Cu at 40 cm SSD The validity of this design concept is dependent upon the energy spectra relative to the energy dependent absorption in bone Spectral measurements for this unit seem not to have been reported and are therefore the subject of the present report

Methods

Spectrometer system The detector chosen for this investigation was a high purity germanium detector (ORTEC Oak Ridge Tennessee) with a sensitive volume 4 25 cm in diameter and 4 76 cm in length Total energy resolution of the system is about 2 0 keV (full width at half maximum) using 1 33 MeV photons Detector efficiency relative to a 3 ×3 (7 5×7 5 cm) NaI(Tl) detector is approximately 10 per cent for 1 33 MeV photons The preamplifier and high voltage filter of the system were mounted on the detector which was cooled to liquid nitrogen temperature to reduce thermal noise The signal was processed by a 4096-channel analyzer and a mini computer with the flexibility of accumulating and storing data and performing spectrum smoothing In addition the multichannel analyzer was interfaced to an external computer for further data analysis

Maximum distance between the roentgen source and detector permitted by the size of the room was somewhat less than 3 m During normal operation the photon fluence rate was estimated to be about

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10^{8} cm^{-2} s^{-1} incident upon the detector at this distance The maximum count rate for the detector was about 10^{4} s^{-1} to avoid distortions due to pulse pile up and dead time The collimator arrangement shown in Fig 1 was used to reduce the count rate to a manageable level Collimator C close to the detector reduces the beam cross sectional area to 0 0014 of the detector cross section Further reduction was achieved by collimator B which blocks part of the source seen by the detector

In this manner the count rate was brought below 10^{4} s^{-1} In all measurements dead time losses were less than 15 per cent and the pile up effect was negligible The measured spectrum of each treatment cone was reproducible from time to time

Shielding around the detector consisted of a lead cylinder of 2 54 cm wall thickness lined on the inside with 0 32 cm copper to attenuate the lead characteristic roentgen rays The rear part of the detector connected to the cooling system was covered with lead sheets In this way the background count rate from scattered photons in the room was reduced substantially

Experimental techniques The collimator arrangement (Fig 1) was aligned using a laser beam First collimators A and D were aligned with the detector and fixed in position Collimators B and C (both having smaller diameters) were then mounted between collimators A and D on two stands with micrometer adjustments horizontally and vertically Using the laser beam again collimators B and C were aligned with A and D Copper was used for collimator C to prevent the generation of lead K roentgen rays and to absorb some lead K rays from the walls of the collimators A and B In a perfect alignment the lead K lines should be reduced to a minimum in the measured roentgen ray spectrum and this fact was always used for final alignment

After the detector and the collimators had been aligned the laser was removed and the head of the unit with the treatment cone was placed in position Since the treatment cone had previously been found to be perpendicular to the tube head levelling the tube head made the central axis of the beam parallel with the horizontal collimation axis The position of the source was centered on the same line by moving the tube head slightly and finding the position for maximum count rate

Spectrum measurements were made using four different treatment cones as well as without any treatment cone The characteristic features of these

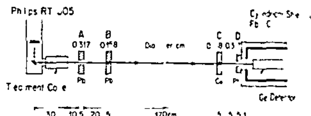


Fig 1 Experimental arrangement for the spectrum measurements

Table 1

Characteristics of the cones used

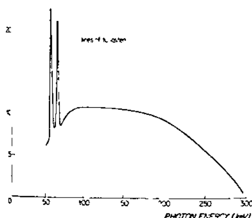
Cone	SSD (cm)	Field size (cm²)	E_{eff} (keV)	Dose rate (Gy/min)
Long A	30	8×10	167	1 01
Medium B	15	8×10	10	0 91
Medium C	10	6×8	40	0 95
Short D	6	3 (dia)	355	0 93

Estimated from half value layer measurements (Cohen et al 1978)

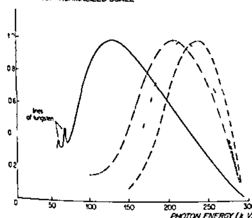
cones are given in Table 1 All cones are designed to deliver approximately the same dose rate at constant kV and current Therefore the shorter cones are more heavily filtered

Corrections The measured data were corrected for scattered photons generated in the room and entering the detector assembly primarily from the rear These photons are multiply scattered and their energies are low Lead of 6 mm thickness was found to be sufficient to prevent most of this radiation from reaching the detector It was difficult to increase this shielding due to the connections to the liquid nitrogen container Counts due to remaining scattered photons of this kind were subtracted using a spectrum measured with a lead block in front of collimator D Scattered photons from the inner walls of the collimators including some characteristic roentgen rays were no doubt present but had been minimized by the careful alignment The choice of copper for collimator C reduced the presence of characteristic roentgen rays from lead Further correction was not applied

Correction for the limited resolution of the detector is unnecessary since it is insignificant in relation to the width of the energy distribution of the bremsstrahlung spectrum The detector counts include partial absorption events as well as those resulting from total absorption of the photons The manufa



ig 2 The roentgen ray spectrum from the RT 305 without treatment cone



ig 3 The roentgen ray spectra from long cone A (——) medium cones B (- -) C (—) and short cone D (— -) The spectra are normalized to 1 0 at maximum

urer s specification of the ratio of the photo peak eight to the Compton plateau height was approxi ately 35 to 1 at ^{60}Co and will be even larger at wer energies Additionally the energies of the ompton edges generally fall below the energies of he main part of the spectrum However in the wer end of the spectrum particularly without any one and with the long cone Compton electrons ve a small contribution (5–10%) The effect on hapes of the spectra were considered too small to otivate an elaborate correction technique which ould have been feasible (LEVY et coll)

The major correction was that for the energy de endent efficiency of the detector i e the fraction f photons incident upon the front surface of the etector that is detected This efficiency factor was measured using calibrated nuclide sources with photon energies ranging from 40 keV to 3 25 MeV The activities of the nuclides were given by the manufacturer (Isotope Products Labs Burbank California) with an average uncertainty of ±4 per cent In the photon energy range from 40 to 300 keV the efficiency as defined was found to decrease from 0 75 to 0 34 These measurements were made with a source to detector distance of 25 cm and without collimators This geometry was different from that used in the bremsstrahlung measurements (Fig 1) Separate experiments were made with the nuclide sources with and without collimation and the effect on the shape of the efficiency versus energy function was found to be negligible It should be noted that only the relative efficiency values are of importance and that absolute measurements of photon fluences were not intended Consequently the measured bremsstrahlung spectra could be corrected with the (arbitrarily normalized) energy efficiency curve obtained with the nuclide sources without collimation

Results

The corrected roentgen ray spectrum from the RT 305 without any treatment cone is given in Fig 2 The two K lines of the tungsten target are prominent at the lower end of the bremsstrahlung spectrum and amount to 10 per cent of the total photon fluence The rather heavy inherent filtration of the tube and its housing (2 5 mm Cu equivalent according to the manufacturer) is obviously attenuating the photons of low energies

The spectra resulting from the 4 cones specified in Table 1 appear in Fig 3 The spectra are all normalized to 1 0 at maximum As can be expected with additional filtration in the treatment cones the peaks of the spectra are pushed to higher energies and the energy range of the spectrum with high filtration becomes narrow A small amount of tungsten K roentgen rays and some low energy photons below 100 keV can still be seen for the long cone A The spectra also show that K rays from the lead collimators and shielding have been effectively removed

Discussion

The fact that correction for partial absorption of the photon energy by Compton scattering was not carried out makes the shape of the spectra at the low

Table 2

Results of experiments and calculations

Cone	Filtration	Measured HVL (mm Cu)	Calculated HVL (mm Cu)	f^{bone}_{muscle}
Long A	4 8 mm Cu	4 0	3 9	1 18
Medium B	1 5 mm Pb	5 ?	5 1	1 04
Medium C	? 2 mm Pb	5 8	5 5	1 01
Short D	3 5 mm Pb	6 1	5 7	1 00

energy end somewhat uncertain Therefore in Figs 2 and 3 the curves were not extended to lower energies than those where the estimated uncertainty is about ±50 per cent As discussed previously this effect quickly vanishes at the higher energies and does not affect the main part of the spectrum Statistical inaccuracies in the spectrum determined by the number of counts per channel were always made negligible (<1%) by accumulating a sufficient number of counts

The spectra are representative for the beam axis defined as identical to the collimator axis No off axis measurements were made The use of collimator B (Fig 1) implies that photons emanating from different portions of the target are not measured with equal efficiency No effort was made to analyse variations across the focus but the possible variations cannot be substantial since measurements after independent alignments were reproducible

As an overall double-check of the spectral measurements the half value layer (HVL) *in copper* calculated from the spectra in Fig 3 was compared with that directly measured in a narrow beam geometry (Table 2) From the measured spectrum N(E) the following formula was used to calculate HVL

$$\frac{D(x)}{D_o}=\frac{\int E\times N(E)\times e^{-\mu x}\times[\mu_{en}/\varrho]\,dE}{\int E\times N(E)\,(\mu_{en}/\varrho)\,dE} \qquad (1)$$

where E is the photon energy x is the added filtration μ is the linear attenuation coefficient of a chosen material (in the present experiments copper) and $[\mu_{en}/\varrho]$ is the mass energy absorption coefficient for air (used since the measured HVL was based on exposure measurements) Eq (1) can be solved numerically for HVL = X_h by setting the dose ratio $D(X_h)/D_o = \frac{1}{2}$

The calculated HVL are 2 to 6 per cent lower for the ? cones (Table 2) Considering the uncertainties in the spectrum measurements (especially for the long cone A) and the difficulty in completely *eliminating high energy scattered photons in the* HVL measurement (especially for the heavily filtered shorter cones) the results agree more or less as well as can be expected

The manufacturer describes the RT 305 as a hard roentgen ray therapy unit with a filtration in the range of 5 mm Cu to 3 5 mm Pb designed to achieve water equivalent absorption in bone In order to confirm this f factor ratios for bone and muscle were calculated

$$f^{bone}_{muscle}=\frac{\int[\mu_{en}/\varrho]^{bone}_{air}\,N(E)\,dE}{\int[\mu_{en}/\varrho]^{muscle}_{air}\,N(E)\,dE} \qquad (2)$$

The results are also listed in Table 2 The ratios of the f factors were found to be very close to one for the heavily filtered cones but are significantly greater for the lightly filtered cones

Conclusions

For the RT 305 roentgen ray therapy unit the spectra are deliberately changed for different treatment cones which in turn are designed to allow somewhat different but limited treatment depths The roentgen ray spectrum from the lightly filtered long cone has a maximum at 150 keV and an f factor for bone 18 per cent higher than that for muscle For the other cones and filtrations the spectra lie primarily above 150 keV with the f factor for bone close to that for muscle

SUMMARY

Bremsstrahlung spectra from a heavily filtered 300 kV therapy unit have been measured using a high purity germanium detector Measurements were made without filtration and with four different filters up to 3 5 mm Pb The photon fluence spectra have maxima at energies ranging from 100 keV to 250 keV Half value layers calculated from the measured spectra agree within 2 to 6 per cent with measured half value layers The effective energy absorption coefficient for bone is shown to be within 5 per cent of that for soft tissue for all but the most lightly filtered beam

ACKNOWLEDGEMENT

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SUPERFRACTIONATED IRRADIATION COMBINED WITH LOW DOSES OF BLEOMYCIN IN THE TREATMENT OF ORAL CARCINOMA

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A combination of irradiation and Bleomycin treatment has been used in the management of many types of tumours with varying results Thus no effect was encountered in the post-operative treatment of *glioblastomas but good results were obtained* in the treatment of penile carcinoma (EDSMYR 1976 ANDERSEN et coll 1981) Also in the treatment of head and neck tumours contradictory results have been reported and in some cases severe local *reaction of the normal tissue occurred* (BERDAL 1976 HANSEN et coll 1976 SHANTA & KRISHNAMURTHI 1976 CACHIN et coll 1977) The results of laboratory experiments also vary indicating differences in the cellular sensitivity to Bleomycin treatment combined with irradiation even in the case of the same cell line when used under different experimental conditions (TWENTYMAN & BLEEHEN 1973 TERASIMA et coll 1975 SAKAMOTO 1978) At present therefore no general conclusions on the effect of a combination of irradiation and Bleomycin can be drawn More basic experiments are needed to suggest different regimes to be tested under clinical conditions However the available data permit some treatment schedules to be defined with a possible control of the local reactions and with *possible improved therapeutic results Thus* some preliminary conclusions may be drawn from the present small clinical series treated with a combination of Bleomycin and superfractionated irradiation

Theoretic conditions

Previously the ideas were described which led to the development of a superfractionated regime in the first hand in order to control the rad ation resistant hypoxic cells in tumours (JAKOBSSON & LITTBRAND 1973 BACKSTROM et coll 1973) This superfractionated regime has now also been used in combination with Bleomycin The cytostatic effect *of Bleomycin has been shown to be* biphasic both with regard to time and dose The survival curve has an upward concave curvature and consists of a steep initial and a less steep terminal portion (URANO et coll 1973 TERASIMA et coll 1976) This indicates *that the cells have an early sensitive and a sub*sequent resistant phase in their response to Bleomycin However the development of the resistance is time limited and the original sensitivity reappears after 1 to 2 hours A repeated treatment with small doses after more than 2 hours may therefore result in a better effect than the effect of a single high dose

A synergistic effect of Bleomycin and irradiation has been demonstrated in different experimental systems It has been shown that Bleomycin interferes with the radiation recovery processes both in asynchronously and synchronously proliferating cells (MATSUZAWA et coll 1972 SHRIEVE & HARRIS 1979) The interference expresses itself in a decrease of the extrapolation number of the survival curves The effect of Bleomycin is therefore dependent on *the radiation* dose being higher after low doses The results of experiments with synchronized Chinese hamster cells showed an enhancement ratio for G cells of 1 17 and 1 06 at the 50 and 1 per cent survival level respectively In principle similar results were obtained with cells also *in the S-phase* (MIDANDER

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Table

Treatment schedules and results in 7 cases of oral carcinoma Classification according to TNM (UICC 1978) and CRE according t… et coll (1971) No correction for RBE or volume has been made Gap correction according to ELLIS *(1969)*

Case No	Age and sex	Site	Size (cm)	Stage	Daily fractions (Gy×times)	Time interval (hours)	Split course interval (weeks)	Total dose (Gy)	CRE	Bleomycin (mg)		Surv… (mont…)
										Daily dose	Total dose	
1	78 M	Lip	7×12	IV	1×3	4 4 16	0	81	19 1	7 5×1	147 5	54+
2	83 M	Lip	3×6	III	1×3	4 4 16	3	80	18 1	7 5×1	195	14
3	74 F	Tongue + lymphogl metastases	3×6+ lymphogl metastases	III	1×3	4 4 16	4	75	16 5	5×1	90	48+
4	78 M	Bucca	4×4	III	1×2	6 18	2	78	17 2	2 5×2	173	29+
5	65 F	Soft palate	3×3	II	1×2	6 18	2+2	74	16 1	2 5×2	145	20+
6	70 M	Bucca	4×5	IV	1×2	6 18	2	76 5	17 1	2.5×2	187 5	18+
7	56 M	Tongue	3×6	IV	1×2	6 18	2	77 5	17 1	2 5×2	147 5	12+

Died of pleural empyema
Local recurrence at 4 months

et coll 1980) It may be concluded that a combination of Bleomycin with small radiation doses may be profitable to take advantage of its sensitizing action This suggests that a superfractionation of the radiation dose in combination with Bleomycin treatment might be of particular advantage

Material and Methods

The material comprised 7 patients 5 males and 2 females between 56 and 83 years (Table) The primary tumour site was the lip the bucca and the tongue in 2 cases each and the soft palate in one case all the tumours being advanced one tumour in stage II 3 tumours each in stages III and IV (TNM classification UICC 1978) All tumours were epidermoid carcinomas The primary tumour and the nearest regional lymph station were included in the target volume The radiation source was a Brown Boveri Betatron (35 MeV) giving a dose rate of 0 7 to 2 5 Gy/min (SSD 110 cm) and a Varian Linear Accelerator (4 MV) giving 2 0 Gy/min (SSD 80 cm) One patient was treated with a combination of electrons and photons and the others with electrons only

The irradiation schedules appear in the Table Irradiation was given 2 or 3 times daily in general in 2 equal courses separated by a time interval of 2 to 4 weeks The total dose was 74 to 81 Gy CRE=16 1 to 19 1 reu Bleomycin was given either in 5 mg or 7 5 mg doses daily one to two hours before the first irradiation (3 cases) or 2 5 mg twice daily one to t… hours before each irradiation (4 cases) The t… Bleomycin dose varied between 145 and 195 mg w… one exception (Case 3) in which the treatment ha… be discontinued due to severe sensitivity reactio…

Results

Total remission of all the 7 oral carcinomas … curred in each case before half of the treatm… course was completed During a period of 7 to … months (Table) no local or distant recurrence … curred except in one case One patient (Case 2) d… of pleural empyema 14 months after the start … treatment At post mortem no residual tumour … metastases were found nor Bleomycin and … fibrosis of the lung tissue A plastic reconstructi… of the lip was made in another patient (Case 1) d… to the great loss of tissue as a result of the la… tumour mass The surgery was performed one y… after the completion of the therapy In the remov… tissue no residual tumour was found and no po… operative complications occurred

In all patients confluent mucositis appeared af… a dose of about 30 Gy i e some time before the fi… treatment course was completed With two exce… tions the mucosal reactions healed during the … week rest period but reappeared before the end … the second treatment course In one of the ca… (Case 3) the local reaction was so intense that t… rest period between the two courses had to be …

-ased while in another case (Case 5) in addition -other 2 week rest period had to be inserted after -Gy Within one month after completion of the -rapy the local reactions faded away in all cases During the observation period no osteitis local fi-rosis or any complication from the lungs were ob-served In Case 7 the locally advanced tumour dis-appeared completely after treatment The tumour -cluded the entire tongue and the floor of the mouth However after 4 months observation a -cal recurrence in the tongue and a lymph node metastasis occurred

Discussion

The severe local reaction of the mucosa and other -rmal tissues has been regarded as the major dis-advantage of a combined irradation and Bleomycin -eatment (SHANTA & KRISHNAMURTHI CACHIN et -oll) This reaction can be attributed to the radia-tion sensitizing effect of Bleomycin on the well -xygenated tissues The present preliminary results -dicate that the severe local reactions can be suf-ficiently well controlled and avoided to a great ex-tent by superfractionated irradiation combined with Bleomycin treatment in a split course schedule in -ne with the theoretic considerations Since the -aterial is still small no definite conclusions regard-ing the therapeutic efficiency of this treatment can -e drawn The absence of any severe local reactions -ay be considered an encouragement to continue -is type of treatment Furthermore the rapid dis-appearance of the tumours and the absence of re-currence may be a good prognostic sign particularly -i view of the observations of MANTYLA et coll -979) The enhanced response may also contribute -a rapid reoxygenation of the tumours (DENEKAMP -977) and thus increase the efficiency of the treat-ment

SUMMARY

Seven patients with advanced oral carcinoma have been given 1 Gy per irradiation up to a total dose of 74 to 81 Gy Bleomycin was given intramuscularly before irradiation The therapeutic ratio of the proposed scheme was good The early reactions were severe but were managed by a split course technique No late complication occurred All patients were tumour free 18 to 54 months after treatment A relapse occurred only in one case The results are not -lusive but bearing in mind that the results in ad-vanced oral carcinoma are poor they suggest that the -ent regime may be promising

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RADIATION THERAPY OF GLOTTIC CARCINOMA STAGE I

B JOSE D L CALHOUN A MOHAMMED and D A TOBIN

Radiation therapy is being accepted as the pri nary treatment modality for early carcinoma of he vocal cords In those patients who fail after adiation therapy cure is possible by surgery The onservation of voice in this early disease which is equently achieved with radiation therapy is a very gnificant point in the management of this disease n order to achieve optimum results the effects of he total radiation dose time dose fractionation and he type of radiation must be considered

Materials and Methods

A detailed retrospective analysis of 321 patients ith a diagnosis of laryngeal carcinoma treated at is department from October 1953 to December 975 was done Ten patients were excluded because ey did not complete the planned treatment Of the maining 311 patients 81 (26%) patients were aged as stage I (T1N0M0) glottic carcinoma using he American Joint Committee (AJC) staging system 1978) All the 81 patients were treated with primary adiation therapy with a curative intent and the esults are now reported

The distribution of the patients by age appears in Table 1 The maximum number of patients was in the age range of 45 to 74 years The sex and race distribution is given in Table 2 Eighty eight per cent of the patients were male and 80 per cent of the total number were white compared with 14 per cent of patients in the black race

After a complete history physical examination and review of systems the patients were evaluated by an otorhinolaryngologist by indirect and direct laryngoscopy The staging included soft tissue radiography of the neck tomography and laryngo graphy All the patients had a microscopic diagno sis of carcinoma The tumors were classified as squamous cell carcinoma in 69 cases and carcinoma without specification of type in 12 cases

The major part of radiation treatment was given using megavoltage radiation since the department had a ^{60}Co unit from 1954 and later by 4 MeV linear accelerator The technique of irradiation was accomplished by using lateral parallel opposed open and wedge fields measuring 4 cm×5 cm or 5 cm×5 cm The radiation dose varied from 59 Gy to 68 Gy and the majority of the patients received a midplane dose of 64 Gy The doses were delivered at a rate of 10 Gy per week in 5 fractions per week

Results

The survival rates were calculated from the last day of irradiation using the actuarial (life table) method The crude and adjusted 5 year survival rates are shown in the Figure In the calculations of the adjusted survival rate those patients who died without disease are withdrawn from the risk of dying from glottic carcinoma The crude 5 year survival rate was 69 per cent (SE 5%) and the adjusted 5 year survival rate as 86 per cent (SE 4%)

Failures Twelve per cent (10/81) of the patients had local recurrence and were further managed by

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surgery or radiation therapy The clinical data on the 10 recurrences appear in Table 3 The median time of recurrence was 25 5 months Eight of 10 re currences (80%) were cured by surgery and the overall local control rate (98%) is given in Table 4

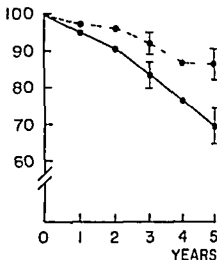
Complications No instance of laryngeal necrosis was encountered in the present series The post irradiation hoarseness and occasional laryngeal edema were controlled in about 2 to 3 months with adequate voice rest and antibiotics

Second malignancies A second malignant tumor developed in 9 patients colon 3 lung 2 prostate hypopharynx oral cavity uterine cervix and skin one each One of these patients had carcinoma of both the colon and the uterine cervix

Discussion

Several large series are reported on the efficacy of radiation therapy in the management of early glottic carcinoma (JAKOBSSON 1973 JØRGENSEN 1974 HJELM HANSEN et coll 1975 1979 JØRGENSEN et coll 1979 LUND et coll 1979) Preservation of the voice is an advantage over surgery In general the results of radiation therapy have a close rela tionship to the mobility of the involved cord WANG (1974) has reported a 5 year recurrence free rate (determinate group excluded those patient dead of intercurrent disease with no evidence of local re currence) of 87 per cent in a series of 325 patients of stage T1N0M0 with normal mobility For a tumor limited to the anterior two thirds of the vocal cord the 5 year NED (no evidence of disease) rate is reported as 92 per cent for lesions of both anterior two thirds and anterior commissure 81 per cent and for lesions of the entire or posterior third of vocal cord 70 per cent respectively Similar differences in the 5 year NED rate have been reported by SUNG et coll (1979) The 5 year NED rate for carcinoma in situ has been 100 per cent and for T1 lesions it is 76 per cent HARWOOD et coll (1979) have reported a 5 year crude survival rate of 74 per cent and a corrected actuarial survival rate of 95 per cent in a series of 333 patients

The superficial carcinoma of the cord is treated up to a dose of 60 Gy in 6 weeks at a rate of 2 Gy per fraction HORIOT et coll (1972) have recommended 65 Gy in 6 weeks for small exophytic tumors and 70 Gy in 6½ weeks for bulky glottic lesions MORRISON & DEELEY (1962) have reported that 70 Gy in 6 weeks was well tolerated for small size portals (25 cm²)



Five year crude (—) and adjusted (---) survival (per cent) rate (life table method)

Table 1

Age distribution at the diagnosis

Age group (years)	No of patients	Per cent
<45	5	6
45–54	20	25
55–64	25	31
65–74	20	25
>75	11	13
Total	81	100

Table 2

Race and sex distribution

Race	Male	Female	Total	Per cent
White	59	6	65	80
Black	7	4	11	14
Not specified	5	0	5	6
Total	71 (88%)	10 (12%)	81	100

but 64 Gy in 6 weeks was the limit of tolerance for larger portals In the present series the majority of patients received 64 Gy in 6 weeks which is similar to the dose used by WANG (1974)

Since the lesions have a tendency to spread towards the arytenoid region a homogeneous distribution of radiation throughout the entire glottic area is required This can be achieved by using lateral opposed open and wedge fields by an e[illegible]

Table 3

Failures after irradiation in 10 cases of glottic carcinoma Median time of recurrence was 25.5 months DWD Died with disease DI Died with intercurrent disease NED No evidence of disease

Dose (Gy)	Time to recurrence (months)	Treatment	Controlled by surgery	Survival from initial treatment (months)	Status on last follow up
63	6	Laryngectomy+left neck dissection	Yes	56	Died of oral carcinoma
60	10	Laryngectomy+irradiation to stoma (50 Gy)	No	29	DWD
65	12	Laryngectomy	Yes	64	Died of colon carcinoma
65	13	Laryngectomy	Yes	79	DI
6	23	Laryngectomy+irradiation (60 Gy)	No	42	DWD
64	28	Laryngectomy	Yes	84	DI
64	34	Laryngectomy	Yes	77	NED
65	49	Stripping	Yes	97	NED
63	65	Laryngectomy+radical neck dissection	Yes	88	NED
65	75	Laryngectomy	Yes	87	NED

Table 4

Local control rate

	No of patients	Per cent
Primary controlled by irradiation	71/81	88
Recurred	10/81	12
Cured by surgery	8/10	80
Overall local control rate	79/81	98

oblique wedge fields or by a combination of these as described by WANG (1976) The technique of radiation therapy in early glottic carcinoma has also been discussed by MOSS et coll (1979) The posterior half of the arytenoid must be excluded from the high dose region by reducing the portals to 50 to 60 Gy in order to prevent excessive arytenoid edema Also to include the tumor extending to the anterior commissure in the field of radiation the anterior border of the beam should extend one centimeter beyond the anterior edge of the thyroid cartilage

The reported failure rates in glottic carcinoma Stage I range from 9 to 21 per cent as described by BOSCH et coll (1978) In the present series the recurrence rate is 12 per cent and 70 per cent of the recurrence occurred within 3 years after radiation therapy which is similar to the results reported by PEREZ et coll (1968) and HARWOOD et coll Both JÖRGENSEN & SELL (1971) and NIEDERER et coll (1977) have discussed causes of failure including cell type clinical stage inadequate size of the portals etc Presence of residual malignant cells in the treated volume is the major cause of the failures The surgical cure rate is high as in the present series (80 per cent) The possibility to cure is dependent on the mobility of the involved cord or the extent of the initial lesion

The continued follow up of these patients is important to diagnose late recurrences and to find second carcinomas as demonstrated in the present series HARWOOD et coll have reported a second malignancy rate of 2 per cent per year half within the respiratory tract They had only 4 cases of carcinoma of the larynx and pharynx which did not have an increased incidence of radiation induced carcinomas Only one case in the present series had a carcinoma of the hypopharynx These data contradict the argument of using surgery as the primary treatment because of radiation induced carcinoma

SUMMARY

A detailed retrospective analysis of 81 patients with a diagnosis of glottic carcinoma stage I (T1N0M0) from 1953 to 1975 has been done Eighty-eight per cent of the patients were male and 80 per cent of the total number

were white The majority of the patients received a dose of 64 Gy in 6½ weeks The crude 5 year actuarial survival rate was 69 per cent and the adjusted 5 year actuarial survival rate was 86 per cent Local recurrence occurred in 12 per cent and of these 80 per cent were cured by surgery The median time of recurrence was 25 5 months No case of laryngeal necrosis occurred and 10 second malignant tumors were diagnosed in the follow up

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FROM THE RADIATION THERAPY SERVICE (DIRECTOR Z PETROVICH) VETERANS ADMINISTRATION WADSWORTH MEDICAL CENTER LOS ANGELES CALIFORNIA 90073 USA

ADVANCED CARCINOMA OF THE TONSIL

Treatment results

Z PETROVICH H KUISK L JOSE R BARTON and D RICE

arcinoma of the tonsil accounts for over 2 per t of all malignant tumors and for 10 per cent of se arising in the upper respiratory and digestive t (TAPLEY et coll 1959) The incidence of car ima of the tonsil has increased sharply in the nt years particularly among patients under the of fifty (SHUMRICK 1975) This disease predomi tly affects males in their sixth and seventh dec of life (SIMONS et coll 1963 SCANLON et coll 3) In 94 per cent of the patients the tumor is a amous cell carcinoma (FAYOS & LAMPE 1971) nor spread into adjacent structures such as the e of the tongue and soft palate is common (TERZ 'ARR 1967 PEREZ et coll 1970)

'he incidence of metastatic tumor in the cervical iph nodes varies from 62 (GARY BOBO et coll 3) to 75 per cent (FLETCHER & LINDBERG 1966) ipsilateral subdigastric node being the one most quently involved The reported incidence of tant metastases varies from 4 (SCANLON et coll) 16 per cent (RIDER 1962) The present report iews the clinical experience with 205 patients th carcinoma of the tonsil who were treated in s Medical Center from 1946 through 1976

Material and Methods

All 205 patients with microscopically proven uamous cell carcinoma of the tonsil who were eated in this Center are included no patient with is diagnosis being excluded

The analysis was accomplished through the review of the records The initial performance status of the patients was evaluated according to the Karnofsky Scale (KARNOFSKY 1949)

Symptomatology microscopic appearances local extent of the tumor metastatic patterns treatment methods and results were analyzed A post mortem examination was obtained in 90 (44%) patients

Staging The patients were retroactively staged using the Clinical Staging System for Cancer of the Head and Neck of the American Joint Committee for Cancer Staging and End Results Reporting (1978)

T1 Tumor ≤2 cm in greatest diameter

T2 Tumor >2 cm to 4 cm in greatest diameter

T3 Tumor >4 cm in greatest diameter

T4 Massive tumor >4 cm in diameter with invasion of bone soft tissues of neck or root of tongue

N0 No clinically positive nodes

N1 Single clinically positive homolateral node ≤3 cm in diameter

N2 Single clinically positive homolateral node >3 cm <6 cm in diameter or multiple homolateral nodes ≤6 cm

N3 Massive homolateral node(s) bilateral nodes or contralateral node(s)

M0 No distant metastases

M1 Distant metastases present

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Stage I T1 N0 M0
Stage II T2 N0 M0
Stage III T3 N0 M0
T1 or T2 or T3 with N1 M0
Stage IV T4 any T with N2 or N M0 and T with any N and M1

Treatment Radiation therapy was the primary treatment in 183 (89%) patients while combined irradiation and surgery was employed in 16 (8%) Combined irradiation and chemotherapy was used on 6 (3%) patients Before 1969 142 (69%) patients were irradiated with orthovoltage roentgen rays primarily with a beam of the following characteristics 250 kV 15 mA 2 5 mm Cu HVL and a TSD with a minimum of 50 cm In most of these patients the primary site was irradiated through five portals which included two lateral opposed one posterior occipital one submental and a boost field through a transoral cone 3 5 cm in diameter The lymph nodes of the neck were not irradiated electively but only when palpable lymph nodes were present The daily dose varied from 150 to 280 cGy Irradiations were given 5 days a week but not all portals were treated each day The total tumor dose ranged from 55 to 65 Gy with the mean dose of 60 Gy over a period of 35 to 50 days In addition 30 of these 142 patientes also received irradiation from interstitial implants ^{226}Ra—7 patients ^{222}Rn—17 patients ^{32}P—6 patients (^{32}P was injected into large primary tumors or lymph nodes before 1960)

From 1969 to 1976 63 (31%) patients were given ^{60}Co teletherapy Opposed lateral portals were used which included the upper cervical lymph nodes Only a few patients were irradiated through a single ipsilateral portal The lower cervical lymph nodes were irradiated through a single anterior field which included the supraclavicular region The patients were irradiated at 80 cm TSD 5 days each week all portals daily The daily tumor dose was 200 cGy and a minimum total dose of 60 Gy was delivered over 42 to 50 days In addition 5 of these 63 patients also received irradiation from interstitial implants ^{222}Rn—2 patients ^{192}Ir—1 patient ^{125}I—2 patients) The lymph nodes of the neck at risk (N0) were irradiated electively

The 205 patients irradiated included 32 who received less than 50 Gy of which 16 patients received less than 40 Gy The reason for these lower doses was palliative treatment in 13/32 pre or post-operative irradiation in 7/32 patients refusal of further irradiation in 5/32 death during the course of treatment in 4/32 while in 3/32 the reason was not apparent

Table 1

Multiple malignant primary lesions (except those of the skin and lip) in 51 patients with carcinoma of the tonsil

Site of origin	No of tumors
Lung	9
True vocal cord	8
Floor of mouth	7
Opposite tonsil	7
Anterior 2/3 of tongue	4
Pyriform sinus	3
Maxillary antrum	3
Soft palate	2
Pancreas	2
Prostate	2
Lymphoma	2
Esophagus	1
Stomach	1
Rectum	1
Carcinoid ileum	1
Total	53

Of the 51 patients 49 had a second primary lesion Additional 2 had 2 primary malignant lesions except the carcinoma of the tonsil Thus these 2 patients had 3 separate primary lesions

Combined surgery and irradiation was employed in 16 (8%) selected patients Of these 10 were given post operative radiation therapy (6 for macroscopic disease and 4 for microscopic residual disease) and 6 received preoperative irradiation before radical neck dissection Combined chemotherapy and irradiation was employed in 6 (3%) selected patients with advanced stage IV tumors Of these 4 received intraarterial infusion of 5 fluorouracil before the beginning of radiation therapy and 2 received high dose Methotrexate intravenously (50 mg/kg) with Citrovorum Factor rescue This was repeated once after two weeks

Patients The age of the patients ranged from 30 to 85 years with the mean age of 59 years 25 (12%) being under 50 years of age Of the 205 patients irradiated 202 were males and 3 females In 40 per cent of the patients the initial performance status (IPS) was less than 80 on the Karnofsky scale

The most frequent pre existing signs and symptoms were sore throat in 129 (63%) patients neck mass in 34 (17%) finding of tumor at the routine

Table 2

Incidence of nodal involvement by site in 137 patients

Location	Ipsilateral (n=137)		Contralateral (n=33)	
	No of patients	Per cent	No of patients	Per cent
Subdigastric	109	79	27	82
Submaxillary	85	62	23	70
Mid-jugular	83	60	24	73
Mid posterior	40	30	10	30
Supraclavicular	20	15	9	27
Submental	16	12	6	18
Upper posterior cervical	14	10	8	24
Lower posterior cervical	12	9	5	15
Pre auricular	3	2	0	0

Table 3

Local extension of the tonsillar carcinoma

Site of extension	No of patients	Per cent
Tonsillar pillars	204	100
Soft palate	191	94
Base of tongue	164	80
Lateral pharyngeal wall	123	60
Retromolar trigone	91	45
Gingiva	77	38
Hypopharynx	71	35
Nasopharynx	67	33
Hard palate	40	20
Floor of mouth	25	12
Mandible	18	9
Anterior 2/3 of tongue	12	6
Larynx	7	3

One patient had disease limited to tonsillar fossa

Table 4

T and N stage distribution in all patients

	T1	T2	T3	T4	Total
N0	8	8	18	34	68
N1	0	4	8	19	31
N2	0	3	10	17	30
N3	0	2	15	59	76
Total	8	17	51	129	205

Includes 1 patient with M1 disease
Includes 4 patients with M1 disease

physical examination in 21 (10%) trismus in 14 (7%) loss of more than 10 per cent of body weight in 8 (4%) bleeding in one and hoarseness in one

Metastatic disease in lymph nodes of the neck was detected in 137 (67%) patients of which 33 (24%) had bilateral involvement Metastatic disease below the clavicle was present in 5 (2%) In 51 (25%) of the 205 patients 53 primary malignancies were detected at sites other than the tonsil Of these 53 sites 34 (64%) were above the clavicle and 19 (36%) were below the clavicle

An additional 11 patients had squamous cell carcinomas of the skin or lip and 7 more patients had basal cell carcinoma of the skin The sites of origin for the multiple primary lesions other than those of the skin and lip are listed in Table 1

The analysis of the distribution of cervical lymph node metastases (Table 2) demonstrates that the ipsilateral subdigastric lymph node was the most frequent site of involvement present in 109 (79%) of the 137 patients who had neck metastases The submaxillary lymph node was the second in frequency found in 85 (62%) patients All 33 patients with contralateral cervical lymph node metastases had also ipsilateral nodal involvement

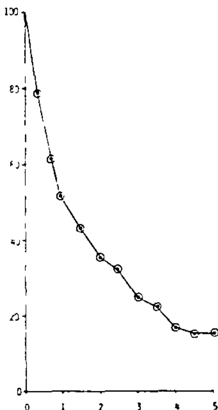
The microscopic grading of the tumor had the following distribution in 205 patients Grande I—1 grade II—58 grade III—117 grade IV—27 In two patients the grade of the tumor was not recorded

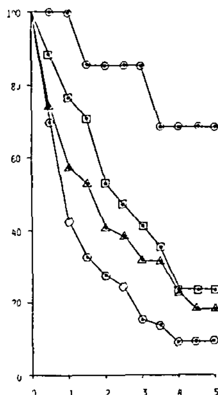
Local extension of the tumor was frequent to the tonsillar pillar and soft palate found in almost all patients (Table 3) Extension to the tongue and lateral pharyngeal wall was next in frequency with 80 and 60 per cent respectively The distribution of the T and N stage appears in Table 4 It revealed that only 4 per cent of the patients were staged T1 8 per cent T2 25 per cent T3 but the majority (63%) presented with advanced stage T4 The N stage distribution shows that 37 per cent of the patients had a massive homolateral bilateral or contralateral (N3) cervical involvement while 33 per cent had no palpable lymph nodes (N0) The remaining 30 per cent was equally divided between those having a single ipsilateral small node (N1) and those with larger or multiple ipsilateral cervical nodes (N2)

Results

The overall 5 year actuarial survival (Fig 1) for all patients was 15 per cent with the median survival of 19 months Detailed analysis of the survival by T and N stage (Figs 2 3) showed a progressive decrease of survival with the increase of the T and N stages however each of these parameters was acting independently

The 5 year survival by clinical staging is given in


Fig 1


Fig 2

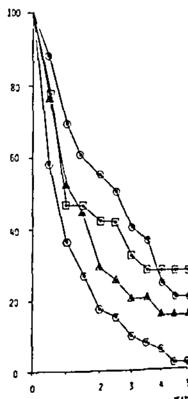

Fig 3

Fig 1 Actuarial survival (per cent) for all 205 patients

Fig 2 Actuarial survival (per cent) by T stage for all patients ⊛ T1 (n=8) ⊡ T2 (n=17) T3 (n=51) ○ T4 (n=129)

Fig 3 Actuarial survival (per cent) by N stage for all patients ⊛ N0 (n=68) ☐ N1 (n=31) ▲ N (n=36) ⊛ N3 (n=6)

Fig 4 It was 69 per cent for stage I and 12 per cent for stage II patients (p=0 006) The 5 year survival for stage III patients was 31 per cent The difference in survival between the stages II and III was not statistically significant (p=0 1) due to the small number of patients in stage II The 5 year survival for stage IV was 9 per cent The difference in survival of stage III and IV patients was significant (p=0 02) The analysis of survival by IPS (Fig 5) showed 22 per cent 5 year survival for patients with IPS >80 against a 6 per cent survival for patients with IPS <80 This difference was significant (p=0 001) and it was independent of the stage of the disease

The analysis of survival by treatment mode (Table 5) showed a 13 per cent 5 year survival for the orthovoltage irradiated patients versus 24 per cent for those given ^{60}Co irradiation (p=0 12) The 5 year survival of patients given combined irradiation and surgery was 24 per cent versus 14 per cent for those irradiated only (p=0 25)

The survival of the 6 patients given irradiation and chemotherapy was poor They had stage IV disease and none of them survived more than 2 years

The 5 year survival of the 25 patients under 50 years of age was 17 per cent versus 15 per cent for the 180 patients over that age (p=0 4)

The current status of all treated patients (Table 6) reveals that a primary tumor control was achieved in 44 (21%) patients The absence of control at the primary site was responsible for the death of 123 (60%) patients Metastatic disease with tumor controlled above the clavicle was responsible for the death of 6 (3%) and intercurrent diseases were the cause of death in 18 (9%) Of these 18 patients 1 died without evidence of tumor Death from malignant disease other than the tonsillar carcinoma occurred in 18 (9%) patients 8 of whom had no evidence of tonsillar carcinoma The number of patients lost to follow up was 35 (17%) which included 20 with persistent tumor and 15 with no evidence of tumor

Metastatic disease below the clavicle (biopsy or autopsy proven) was found in 44 (21%) patients Of

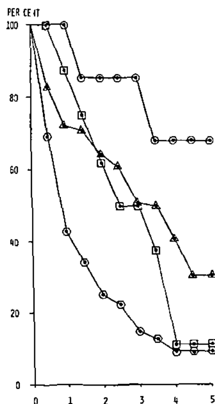


Fig 4 Actuarial survival (per cent) by stage grouping for all patients ⊛ stage I (n=8) ☐ stage II (n=8) △ stage III (n=29) ⊘ stage IV (n=160)

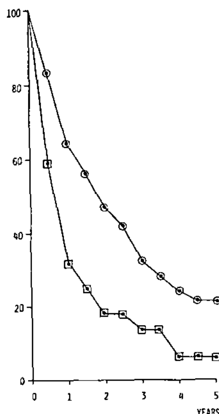


Fig 5 Actuarial survival (per cent) by initial performance status for all patients ⊛ >80 (n=122) ⊡ <80 (n=83)

these 6 had no evidence of tumor above the clavicle

Detailed analysis was performed of the failure in 68 NO patients Among the 52 NO stage III and IV patients 9 (17%) had their primary site controlled and did not develop metastases in the cervical lymph nodes

Seventeen (33%) patients developed a recurrence at the primary site with no evidence of neck node metastases In 26 (50%) the failure at the primary site coincided with the development of cervical lymph node metastases

Among the 16 NO stage I and II patients 9 (56%) had their primary site controlled and did not develop metastases in the cervical nodes In 2 (13%) patients the disease recurred at the primary site but there was no evidence of neck node metastases In 5 (31%) patients the failure at the primary site was followed by cervical metastases

Surgical cure was attempted in 8 patients who had failed at the primary radiation therapy In 6 of these the failure occurred in the neck only and in 2 in the neck and the primary site The surgical attempt to cure was successful in 2 who survived 5 years or longer In the remaining 6 patients the tumor recurred shortly after surgery

Complications Treatment complications of major importance occurred in 17 (8%) patients The most frequent major complication was osteonecrosis of the mandible occurring in 14 patients All of these had an advance stage IV disease with the tumor invading the mandible The treatment consisted of conservative management in 12 and surgical resection in 2 Of these 14 patients with osteonecrosis one recovered fully and was free of tumor at 5 years An additional 3 patients developed post-operative complications two flap necrosis and one flap necrosis with fistula These 3 patients subsequently died of massive local tumor recurrence

Discussion

A series of 205 patients with squamous cell carcinoma of the tonsil was irradiated The main difference between this series and other reports (RIDER BALLANTYNE & FLETCHER 1965 PIERQUIN et coll 1966 TERZ & FARR MATAR & MCCARTEN 1973) is the extent of the disease at diagnosis with the present patients having more advanced stages

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RADIATION THERAPY OF NASOPHARYNGEAL CARCINOMA

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lignant tumors of the nasopharynx are frequent ina particularly in the coastal provinces south : Yangtze River which is in marked contrast the situation in Europe and North America : such tumors are rare (CAMMOUN et coll However during the years before the Libera radiation therapy was used only on a very small in China and the results were poor (LIU 1954) intil after 1949 was radiation therapy seriously dered In the following years irradiation of pharyngeal carcinoma was made available to a ily increasing number of patients Thus at this tment a total of 6660 cases of nasopharyngeal ioma were treated from 1955 to 1973 In the ie of the past 30 years the authors have had e opportunity to investigate the radiothera c aspects of carcinoma of the nasopharynx d mainly on retrospective analyses of some of ases a few points of special interest are now ented

Clinical staging

though the UICC has suggested a TNM clas ation and clinical staging method for this type imor it has not been universally adopted by ors here and abroad for various reasons Since method of staging is intended to relate the extent he disease to the prognosis after treatment an ysis of the influence of various clinical factors urvival was performed by CHANG et coll (1965) s resulted in the criteria shown in Table 1 which ears to reflect the prognosis more closely than se previously used and thus has been used also in present report

The Fifth Chinese National Conference on Naso pharyngeal Cancer at Changsha (1979) suggested some minor modifications in the method of staging mentioned especially as regards whether a primary tumor less than 0 5 cm in diameter but still involving two walls of the nasopharyngeal cavity should be considered as T1 or T2 The final decision awaits the collective results of a nation wide investigation which is under way

When metastatic neck nodes are larger than 8 cm in diameter or although not very large but appear in the supraclavicular region there is a direct cor relation with the frequency of distant metastasis which naturally results in poorer prognosis (cf Table 10)

Results of treatment

Each year since Liberation the 5 year survival rate of nasopharyngeal carcinoma treated by irradia tion at this department has risen steadily (Table 2) Although surgery has been advocated intermittently the results have not been satisfactory Only very early cases have been found suitable for surgery and postoperative irradiation was still needed in most of the cases operated upon As cases of nasopharyngeal carcinoma stage I given radiation therapy alone (Table 2) now have a 5 year survival rate of a good 93 per cent surgery in its present mode is not indicated as the primary type of treat ment

Of the 838 cases treated from 1955 to 1960 the overall 5 year survival rate for all cases was 27 7 per cent increasing to 42 8 per cent for the 1914

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cases treated from 1961 to 1967 and 54 per cent for the 511 cases treated in 1973 (Table 2)

This gradual improvement may have several causes The first factor to discuss is whether the relative proportion of early and late cases has changed in the intervening years Table 3 indicates that no significant change has appeared for the 3 263 cases analysed Cases belonging to stage III and IV have always predominated being around 75 per cent of all cases treated in the different periods

Patients with extensive nodal disease rarely survive 5 years after treatment according to WANG & MEYER (1971) nor do patients with erosion of the base of the skull (HOPPE et coll 1976) However even for such cases in stage IV the 5 year survival rate has increased from 16 5 to 32 per cent in the present series (Table 3)

At present the 5 year survival rate for stage I is 93 3 and for stage II 74 7 per cent It is thus evident that without any change in the principles of irradiation the overall survival rate was markedly improved simply by detecting and treating the cases of nasopharyngeal carcinoma in their earlier stages Some progress has been made in this direction recently by the use of serum VCA IgA antibody enzyme labelled immunoassay as part of the clinical examination (LIU et coll 1979) This is often the case when a neck lymph node has been proved by biopsy to be metastatic carcinoma with no apparent primary tumor in the nasopharyngeal cavity In such instances careful examination of the nasopharynx will sometimes reveal a very small lesion which has been missed at the first examination Some preliminary results indicate the great value of so-called blind biopsy i e taking a biopsy from the ipsilateral Rosenmuller fossa when a VCA IgA test is strongly positive although no tumor is apparent clinically

Table 1

Clinical staging for nasopharyngeal carcinoma

Stage	Criteria	TNM
I	Primary tumor limited to one of the nasopharyngeal walls no palpable cervical lymph nodes	T1 N0
II	1 Primary tumor invading two walls	T2 N0
	2 Unilateral or bilateral cervical nodes mobile diameter not exceeding 8 cm	N1 (T1 T2)
III	1 *Primary tumor extending outside* nasopharyngeal cavity into posterior nasal cavity oropharynx sphenoids middle ear	T3 (N0-N1)
	2 Cervical nodes partly or completely fixed but diameter less than 8 cm	N (T1 T3)
IV	1 Cranial nerve involvement with or without destruction of base of skull	T4 (N0-N4)
	2 Size of cervical nodes exceeding 8 cm	N3 (T1 T4)
	3 Presence of supraclavicular node	N4 (T1 T4)
	4 Distant metastases	M

Principles and technique of radiation treatment of nasopharyngeal carcinoma

Radiation source It is now accepted that ^{60}Co teletherapy units or their equivalent the 4–6 MeV Linear Accelerators are the most suitable machines for irradiation of nasopharyngeal carcinoma The analysis of the 2752 cases treated from 1955 to 1967 from the viewpoint of the radiation source used (Table 4) substantiates this opinion but some points merit attention

The better depth dose distribution from ^{60}Co teletherapy units as compared with conventional irradiation was an important factor in improving the survival rates (Table 4) However when the dosage by conventional irradiation to the primary tumor had been supplemented by intracavity radium mold treatment the results were much improved These results corroborate the principle of radiation therapy that adequate dosage has to be concentrated in a small volume of tissue in the region of the primary tumor to achieve long survivals without harmful sequelae The combination of ^{60}Co teletherapy with intracavitary radium in a small group of patients, confirmed the value of such a technique in improving the survival rates considerably (Table 5)

A suitable after loading intracavitary technique using small ^{60}Co sources is now under consideration which may be expected to further improve the 5 year survival

However one thing should be noted—the combination of external and intracavitary irradiation in stage IV has not improved the results significantly This may be due to the fact that such cases usually have involvement of the cranial nerves with or without destruction of the base of the skull and the radium mold treatment could not cope with such extensive lesions Only an adequate tumor dose from external irradiation with careful localization may increase the survival

Table 2

Five year survival rates for nasopharyngeal carcinoma treated by irradiation in different periods

Stage	1955 to 1960			1961 to 1967			1973 only		
	No of cases	Alive after 5 years	Per cent survival	No of cases	Alive after 5 years	Per cent survival	No of cases	Alive after 5 years	Per cent survival
I	51	33	64 7	75	59	78 7	15	14	93 3
II	168	63	37 5	384	252	65 6	99	74	74 7
III	364	94	25 8	796	329	41 2	278	150	54 0
IV	255	42	16 5	659	172	26 1	119	38	31 9
Total	838	232	27 7	1 914	812	4? 4	511	276	54 0
			p<0 01			p<0 01			

Most of the cases in this period were given conventional irradiation whereas after 1960 ^{60}Co teletherapy was used for the primary nasopharyngeal tumor

Table 3

...e proportions of early and late cases in different periods

1955 to 1960		1961 to 1967		1973 only	
No of cases	Per cent	No of cases	Per cent	No of cases	Per cent
51	6 1	75	3 9	15	2 9
168	20 0	384	20 1	99	19 4
364	43 4	796	41 6	278	54 4
255	30 4	659	34 4	119	23 3
838	100 0	1 914	100 0	511	100 0

Table 4

...des of irradiation and 5 year survival rates for all stages between 1955 and 1967

	Total No of cases	Alive	5 year survival (per cent)	
...entional roentgen ...(180 kV)	308	67	21 8	
...tgen rays plus ...acavitary radium	291	94	3? 3	p<0 01
...tgen ray rotation ...apy (2 0 kV)	107	37	34 6	
...teletherapy	1 976	798	40 4	
...teletherapy plus ...acavitary radium	70	48	68 6	p<0 01

Dosage The question of optimum dosage to the primary nasopharyngeal tumor should be viewed not only from the standpoint of years of survival but also from that of the condition of health of the surviving patients Since most of the patients with stage I and II surviving for more than 5 years have a very good chance also to survive for over 10 years the problem of late radiation sequelae should be kept in mind when treating these patients At present some authors consider that in order to obtain complete eradication of a tumor irradiation should be carried out to the limit of tolerance of the adjacent normal tissues However the opinion at this hospital is that pushing normal tissue dosage to the limit of tolerance is of doubtful value in increasing long term survival rates while the incidence of serious sequelae is inevitably augmented Table 6 indicates that for most cases a dose of 55 to 60 Gy in 6 weeks is quite sufficient to control the primary tumor and a higher dose does not increase the survival rate significantly

The type of the nasopharyngeal malignant tumor has some influence on the dose that should be given For anaplastic carcinoma lymphoepithelioma and poorly differentiated squamous cell carcinoma constituting 85 per cent of the present cases a dose of 50 to 60 Gy is quite adequate The more infrequent well differentiated squamous cell carcinoma may need a slightly higher dose 62 to 65 Gy and some adenocarcinomas may be very resistant to irradiation Also large bulky tumors would require the higher dosage

Table 5

Combination of ⁶⁰Co teletherapy and intracavitary radium in the treatment of nasopharyngeal carcinoma (1961–1967)

Stage	No of cases	Alive	5 year survival (per cent)
I	9	9	100 0
II	76	0	76 9
III	8	17	60 7
IV	7	[illegible]	28 6
Total	70	48	68 6

Table 6

Relationship of dosage to 5 year survival rates in nasopharyngeal carcinoma (1955–1967) Sixteen cases whose radiation schedule was irregular have not been included

	Dose (Gy)			
	30–	40–	50–	60–
No of cases	36[illegible]	259	1 008	1 107
Alive	98	8[illegible]	406	450
5 year survival (per cent)	27 1	31 7	40 3	40 7

Table 7

Subsequent occurrence of cervical metastases in N0 cases with and without elective irradiation of the neck (1955 1967)

Size of primary tumor	Elective irradiation omitted			Elective irradiation given		
	No of cases	Subsequent cervical node metastases	Per cent	No of cases	Subsequent cervical node metastases	Per cent
T1	15	0	0	111	4	3 6
T2	36	8	22 2	149	11	7 4
T3	7	[illegible]	28 6	23	1	4 3
T4	36	6	16 7	64		3 1
Total	94	16	17 0	347	18	5 [illegible]

p<0 005

Technique of irradiation The total dose to the primary tumor is given through two opposed preauricular portals (receiving 2/3 to 4/5 of the total dose) and two angulated malar portals (receiving 1/3 to 1/5 of the total dose) The former usually measure 5 cm×7 cm or 5 cm×6 cm and the latter 4 cm×5 cm The size of the preauricular fields is enlarged forwards to 6 cm width if the tumor has invaded the posterior nasal orifices and downwards to 8 to 9 cm if the tumor extends down to the oropharynx The two malar portals are replaced by a frontal nasal portal or by more angulation inwardly of the beam to the malar portals in the case of invasion of the posterior nasal cavity A postero auricular portal may sometimes be used for cases with cranial nerve involvement or erosion of the base of the skull

In contrast to metastatic neck nodes of other kinds of head and neck carcinomas it has been shown that it is possible to cure those of nasopharyngeal carcinoma with irradiation The entire chains of lymph nodes of both sides of the neck were first irradiated by ⁶⁰Co through tangential frontal fields followed by direct conventional irradiation The total dose to the involved side of the neck amounted to approximately 50 Gy in 6 weeks

It should be emphasized that elective irradiation to all cervical regions not involved clinically (N0 cases) should be considered Table 7 indicates the usefulness of such irradiation especially in the cases with large primary tumors (T3) The probability of a metastatic node appearing later is much lowered when elective irradiation has been given A tissue dose of 35 Gy was usually given in such cases

Cause of death

An analysis of the causes of death in patients treated by irradiation is of value as a guide to further improvement of the survival rate Table 8 shows the known causes of death in 187 of the 235 cases who died within 5 years after treatment given in 1973

It is apparent that distant metastasis predominates as the cause of death The rate of distant metastases

Table 8

of death following irradiation of nasopharyngeal car (1973) Forty eight cases whose cause of death is un known have been excluded

	No of cases	Per cent
currence of primary tumor	45	24 1
nce in the neck	20	10 7
netastases	99	52 9
primary tumor	4	2 1
on myelitis	3	1 6
debility	10	5 4
ıcerous causes	6	3 2
	187	100 0

Table 9

ship between extent of primary tumor and incidence of distant metastases in N0 patients (1973)

	Extent of primary tumor				Total
	T1	T2	T3	T4	
cases	15	47	39	26	127
stant ases	0	1	6	4	11
t	0	2 1	15 4	15 4	8 7

Table 10

ship between si e of cervical nodes and incidence of metastases in patients with nasopharyngeal carcinoma (1973)

	Diameter					Total
	None	1 cm	3 cm	5 cm	8 cm	
cases	127	98	131	120	35	511
istant tases	11	19	26	33	14	103
nt	8 7	19 4	19 8	27 5	40 0	20 2

5

%) in the cases who died is much higher than reported by CHEN (1971) but similar to that in Kwangtung Province (45 5% Chung San ical College 1974) At autopsy TEOH (1957) d 15 cases of distant metastases (48 4%) in 31 s with nasopharyngeal carcinoma The preven of distant metastases in nasopharyngeal carcinoma as in other malignant tumors is thus an important but difficult problem that urgently needs to be solved Theoretically routine adjuvant chemotherapy after irradiation may be of some effect but that awaits confirmation by a controlled clinical trial in a large number of patients The present material shows that the most important measure to be taken is as expected early diagnosis followed by immediate treatment If the primary tumor as well as its neck metastases are treated in their earlier stages the incidence of distant metastases can be greatly reduced (Tables 9 10) thereby increasing the survival rate

Comparison of Tables 9 and 10 shows that the presence and especially the size of the cervical nodes is more important than the extent of the primary lesion as regards the incidence of distant metastases occurring after irradiation BERGER & FLETCHER (1971) came to the same conclusion

The second important cause of death is local recurrence This is perhaps due more to geographic miss than to general underdosage as recurrence in the nasopharyngeal cavity often occurs at a site different from the original one However some nasopharyngeal tumors have shown an unexpected resistance to radiation a small but marked residual mass may remain after full dose external irradiation In such cases a further boosting dose may be required to eradicate the residual tumor This is best given using intracavitary radium local excision by surgery may also be considered instead Attempts to increase the external irradiation to a dose of 70 Gy or more will increase the incidence of late complications

The third and less important cause of death is due to recurrence of the neck nodes after irradiation Although not often occurring it is a rather difficult situation as this kind of recurrence usually occurs in a tumor bed markedly altered by radiation fibrosis with atrophic overlying skin

Concluding remarks

The results gained at this hospital with radiation therapy of malignant nasopharyngeal tumors one of the most frequent malignant tumors in China indicate that all possible efforts should be made to improve the methods for early diagnosis As soon as the diagnosis has been established irradiation should be given as the primary treatment

SUMMARY

Carcinoma of the nasopharynx is one of the most frequent malignant tumors in the southern parts of China A total of 3263 cases treated with irradiation in the course of the thirty years since the Liberation have *been analysed retrospectively The 5 year survival rate* has risen from 27 7 per cent for cases treated during 1955 to 1960 to 54 per cent for those treated in 1973 The technique of irradiation is described emphasizing the importance of suitable techniques for the treatment of the cervical lymph nodes The value of elective irradiation of cervical regions not involved clinically is elucidated Finally the causes of failure of treatment are discussed

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FAST NEUTRON TELETHERAPY FOR ADVANCED CARCINOMA OF HYPOPHARYNX AND SUPRAGLOTTIC LARYNX

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In selecting a therapeutic approach to the treatment of carcinoma of the hypopharynx and larynx consideration must be given to the preservation of voice and the minimization of aspiration problems Hence in many centers definitive radiation therapy is the treatment of choice for early lesions and excellent rates of local control have been achieved (GOFFINET et coll 1973 WANG 1973 GOEPFERT et coll 1975 NASS et coll 1976 HARWOOD et coll 1979) However more advanced lesions are difficult to control with either conventional photon radiation alone (GOFFINET et coll WANG INOUE & SHIGEMATSU 1976 NASS et coll) or in combination with surgery (GOEPFERT et coll MARKS et coll 1978) Some authors advocate using higher total doses of conventional radiation (KARIM et coll 1978 STRYKER et coll 1979) while others (HARWOOD & TIERIE 1979) show that the risk of major complications increases once an NSD of 2 050 ret is achieved and that at least for early glottic lesions the dose response rate is fairly flat above 1 650 ret The difficulty in achieving good local control using conventional photon radiation for large tumor masses may in part be due to a significant hypoxic cell population which is fairly resistant to low linear energy transfer (LET) modalities This accounts for the current interest in high electron affinic radiation sensitizers such as misonidazol and in the high LET radiation modalities such as neutrons pi mesons and heavy ions (WITHERS 1973)

The results of a Phase I preliminary trial at this University are now reported in which fast neutrons from a cyclotron designed for physics research purposes were used to treat patients with advanced squamous cell carcinoma of the hypopharynx and supraglottic larynx This work is part of a larger test of the use of fast neutron radiation therapy for advanced carcinoma of the head and neck region The present report completes a site by site analysis of this work with the results for the oropharynx (GRIFFIN et coll 1979 LARAMORE et coll 1979) and the oral cavity and soft palate (LARAMORE et coll 1970) having been reported elsewhere The majority of the patients in the present series had advanced cervical adenopathy as well as large primary lesions but here only the control at the primary site will be considered The control of advanced cervical adenopathy with fast neutron irradiation has been described elsewhere (GRIFFIN et coll 1978)

Methods

The patients were treated with fast neutrons produced with the University of Washington physics

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cyclotron The beam characteristics of this machine have been described in considerable detail elsewhere (WOOTTON et coll 1975 WEAVER et coll 1977) and so will be only summarized here The neutron beam is produced by accelerating deuterons to ~ 22 MeV and then impacting them on a beryllium target This produces a relatively broad energy distribution with the mean energy being ~ 8 MeV

Only a single horizontal port was available for biomedical work and so the patients were treated in a specially designed chair which could be accurately positioned in front of the port Individual treatment fields were shaped using collimators made from a water impregnated plastic A photon contaminate in the beam is caused by neutron nuclei interactions and this varies with target depth (WEAVER et coll) It is less than 10 per cent at a depth of 10 cm but is implicitly included in the term $cGy_{n\gamma}$ ($rad_{n\gamma}$) Patients were treated at a source skin distance of 150 cm and at this distance the treatment dose rate was ~40 $cGy_{n\gamma}$/min For typical field sizes used in the present work the 50 per cent isodose line in a tissue equivalent phantom lies at a depth of ~ 9 cm at beam center Hence the neutron beam is somewhat less penetrating than ^{60}Co radiation

Before beginning clinical trials the relative biologic effectiveness (RBE) of the beam compared with ^{60}Co radiation was estimated using various animal models (GERACI et coll 1974 1975 NELSON et coll 1975) The actual RBE obtained depended on the fractionation scheme the tissue type and the endpoint chosen Based upon the available data at the time of the clinical trials an RBE = 3 was chosen for use in setting the original neutron dose levels and in relating dosages to photon dosages previously used for comparable tumors The original animal experiments used relatively small numbers of fractions and based upon the reduced probability for repair using high LET radiation i e much smaller shoulder on the cell survival curve the more highly fractionated schemes could correspond to higher RBE values Another point that is obvious in retrospect is that since the neutron beam deposits about 85 per cent of its energy via a knock-on process where a fast neutron transfers a large fraction of its energy to a proton the energy deposition process would be more efficient in tissues with a high hydrogen content such as adipose and neural tissue Using the calculated energy deposition in muscle tissue as a baseline clinical experiences thus far point to an RBE >3 for a late neural tissue dama (LARAMORE et coll 1978 1979) but no unexpect complications occurred in the present work

Various treatment fractionation schemes we used in keeping with somewhat limited access to t cyclotron for medical purposes In an attempt standardize these schemes 300 $cGy_{n\gamma}$ (about 9 photon cGy equivalent) per week were given each Three treatment schedules were investigat for treatments utilizing neutrons alone (1) 1 $cGy_{n\gamma}$ on Mondays and Fridays (2) 100 $cGy_{n\gamma}$ (Mondays–Wednesdays–Fridays and (3) 75 cGy on Mondays–Tuesdays–Thursdays–Fridays At fi patients were arbitrarily assigned to either the or 3 neutron fractions per week schedule but late the 4 neutron fraction per week schedule w adopted as standard by all neutron programs in t United States In the present series as well as in others from this institution (GRIFFIN et coll 197 1979 LARAMORE et coll 1978 1979 1980) no d ference among these schemes was found either terms of local control rate or in terms of adver effects Laboratory examinations at this institutic by NELSON et coll and RASEY et coll (1977) lat suggested an enhanced therapeutic ratio for 2 ne tron fractions plus 3 photon fractions per week mixed beam (neutron/photon) treatment arm w added in order to test this in a clinical setting which 60 $cGy_{n\gamma}$ were given on Mondays–Friday and 180 cGy_{γ} of ^{60}Co radiation were given on Tue days–Wednesdays–Thursdays Patients were treat using a shrinking field technique as described b FLETCHER (1973) The spinal cord dose was limite to <1500 $cGy_{n\gamma}$ on the neutron only regimes and <600 $cGy_{n\gamma}$ + 2700 cGy_{γ} on the mixed beam r gime In general the primary and any area of ma sive adenopathy received between 1700 and 27 $cGy_{n\gamma}$ on the neutron-only regimes and betwe 840 and 900 $cGy_{n\gamma}$ + 3780 to 4140 cGy_{γ} on t mixed beam regime In the early phase of the tri 3 patients with laryngeal tumor and 3 patients w hypopharyngeal tumor who were treated with ne trons alone did not receive radiation therapy to t clinically uninvolved lower neck and supracla icular areas while 2 patients with hypopharynge tumor received prophylactic neutron radiation these areas The remainder of the patients receive between 4500 and 5000 cGy_{γ} ^{60}Co radiation these regions using a single anterior field In genera a small midline block was used in the anterior fie at its junction with the upper neck fields Howeve

this would have compromised the treatment then midline block was used and the field junction was ned every 2 weeks The treatment fields were set using either a Northwest Medical Physics Cas de simulator or a General Electric orthovoltage it and confirmed with beam films taken on the clotron or megavoltage treatment unit Patients ceiving photon radiation were treated in the usual one position while the neutron radiation was de ered with the patient sitting as mentioned

Material

Hypopharyngeal tumors The usual conven n of defining the hypopharynx as extending m the plane of the hyoid bone to the plane of lower border of the cricoid cartilage was fol ved It includes the pyriform sinuses the post coid area and the lower portion of the pos ior pharyngeal wall Twenty patients with ad nced hypopharyngeal tumor were treated with t neutrons during the period from October 1973 ough August 1977 Of these one patient received eoperative mixed beam (neutron/photon) radia n of 3 060 cGy_{γ} + 600 cGy $_{\gamma}$ ~ 4 860 cGy equi lent one received 1 450 $cGy_{n\gamma}$ neutron radiation e received postoperative mixed beam irradiation 2 880 cGy_{γ} + 540 $cGy_{n\gamma}$ ~ 4 500 cGy equivalent d one patient received a 600 $cGy_{n\gamma}$ neutron boost the primary after receiving 5 040 cGy_{γ} mega ltage photon therapy The patient who received mixed beam preoperative radiation therapy had cellent tumor regression and only carcinoma in u was found in the operative specimen She died ease free at the primary site 16 months after the eration but had also developed pulmonary meta ses The patient who received preoperative utron only radiation died disease free 15 5 months ter surgery but in the immediate postoperative riod developed a pharyngeal cutaneous fistula he patient receiving postoperative mixed beam diation developed a pharyngeal cutaneous fistula nd died 10 days after completing radiation therapy he patient who received the neutron boost was ought to have had a complete regression of his imary and neck nodes (T4N3) after completing eatment but then developed a local recurrence 3 onths afterwards He underwent a salvage surgical rocedure but had tumor present at the surgical margins and developed a second clinical recurrence months after operation He died of progressing tumor one year after completing the irradiation The complications of combined surgery and neutron radiation for head and neck carcinoma have been discussed in detail by GRIFFIN et coll (1979) and will not be extensively dealt with here These 4 patients do not fit into the scope of the present analysis and will not be considered further This leaves 16 pa tients with hypopharyngeal tumor treated for cure with neutron or mixed beam teletherapy who con stitute the material for this phase of the investiga tion

Of these 16 patients 10 were treated with neu trons alone and 6 were treated with the mixed beam regime For the mixed beam group the mean age was 60 1 years (range 59-69) with the male female ratio being 5 1 while for the neutron-only group the mean age was 64 3 years (range 45-75) with the male female ratio being 8 2 In almost every case the patients presented with a significant history of ethanol and tobacco abuse All patients underwent a direct endoscopic examination under anesthesia and had biopsy proven squamous cell carcinoma In the neutron only group one tumor was well differen tiated 6 tumors were moderately differentiated and 3 were poorly differentiated In the mixed beam group 5 tumors were moderately differentiated and one tumor was poorly differentiated The lesions were staged according to the recommendations of the American Joint Committee for Cancer Staging and End Result Reporting which is shown in ab- breviated form in Table 1 The distribution of hypo pharyngeal lesions according to this staging system appears in Table 2 *It is important to note the ad* vanced primary and nodal disease status of the pa tients Such an advanced group of tumors is char acteristic of Phase I preliminary trials but makes it quite difficult to compare results with series pub- lished in the literature The pyriform sinus was mas sively involved with tumor in 9 of the patients treat ed with neutrons alone and in 3 of the patients treat ed with mixed beam regime Also one patient in the mixed beam group had a T2 second primary in the oral tongue at the time of presentation The neu trons only and mixed beam groups are not strictly comparable and will be considered separately in the remainder of the report

Supraglottic laryngeal tumors The usual conven tion of dividing the larynx into 3 regions was fol lowed (1) the supraglottis which consists of the epiglottis the aryepiglottic folds the arytenoids and the ventricular bands with its inferior margin

being a horizontal plane passing through the ventricular apex (2) the glottis consisting of the true vocal cords (including the anterior and posterior commissures) with the inferior margin being a horizontal plane one cm below the ventricular apex and (3) the subglottis which is the region extending from the lower glottic margin to the lower border of the cricoid cartilage A total of 11 patients with advanced laryngeal tumors were treated with fast neutrons during the period from March 1975 through December 1976 Of these 2 patients received only preoperative neutron beam irradiation one patient received only a neutron boost of 500 $cGy_{n\gamma}$ after having received 5040 cGy_{γ} conventional photon irradiation and one patient declined further neutron treatments after having received only 120 $cGy_{n\gamma}$ in 2 fractions The patient receiving the neutron boost had no local control of tumor and died 3 months after completing treatment The patient who received only 2 neutron treatments was given an additional 5895 cGy_{γ} with megavoltage photons at another institution and apparently was disease free 3 months after completing therapy at which time he was lost to follow up One of the preoperative patients received 1400 $cGy_{n\gamma}$ to the primary and upper neck and 4500 cGy_{γ} photon radiation to the lower neck and supraclavicular region He had severe wound healing problems and died of a carotid artery rupture The other preoperative patient received 1475 $cGy_{n\gamma}$ to the primary and upper neck and 4950 cGy_{γ} photon irradiation to the lower neck and supraclavicular area At the time of surgery he was found to be disease free at the primary site but had a residual tumor in the cervical nodes (original staging T3N2) He developed a pharyngeal-cutaneous fistula after surgery and had some aspiration problems He was lost to follow up 15 months after surgery and at that time had no tumor in the neck but did have widespread metastatic disease Two other patients received only neutron radiation to areas of cervical adenopathy at times of 5 and 12 months respectively after having had definitive local photon radiation therapy and a surgical resection of the laryngeal primary This leaves 6 patients with advanced laryngeal tumor treated for cure with neutron or mixed beam teletherapy who constitute the material for the present report

Of these 6 patients 5 were treated with neutrons alone and one was treated with the mixed beam regime The mean age of these patients was 60 5 years (range 35–86) with the male female ratio being

Table 1

T and N staging system for hypopharynx

T-staging
- T1 Tumor confined to site of origin
- T2 Tumor extends to adjacent site or region without fi[x] of hemilarynx
- T3 Tumor extends to adjacent site or region with fixat[ion] hemilarynx
- T4 Massive tumor invading bone or soft tissues of nec[k]

N staging (also applicable for larynx)
- N0 Clinically negative nodes
- N1 Single homolateral node <3 cm in greatest dimensi[on]
- N2 Single homolateral node 3–6 cm in greatest diamete[r] multiple homolateral nodes <6 cm in greatest dia[meter]
- N3 Massive homolateral nodes >6 cm in greatest diam[eter] bilateral or contralateral nodes

Table 2

Number of patients with hypopharyngeal tumors having a stage lesion

	Neutrons only (10 patients)					Mixed beam (6 patie[nts])				
	T1	T2	T3	T4	Total	T1	T2	T3	T4	[illegible]
N0	0	0	0	0	0	0	0	0	0	[illegible]
N1	0	0	2	0	2	0	0	0	0	[illegible]
N2	0	1	1	0	2	0	0	1	0	[illegible]
N3	0	2	2	2	6	0	3	2	0	[illegible]
Total	0	3	5	2	10	0	3	3	0	[illegible]

Table 3

T staging system for supraglottic larynx

- T1 Tumor confined to site of origin with normal cord mobility
- T2 Tumor involves adjacent supraglottic sites or glottis without cord fixation
- T3 Tumor limited to larynx with cord fixation or extension to hypopharynx or pre-epiglottic space or both
- T4 Massive tumor extending beyond larynx to involve oropharynx soft tissues of neck or thyroid cartilage

4 2 Almost every case had a significant histor[y of] ethanol and tobacco abuse All patients underwe[nt] direct endoscopic examination under anesthesia [and] had biopsy proven squamous cell carcinoma [The] patient treated with the mixed beam regime ha[d a] poorly differentiated lesion while in the gr

Table 4

Number of patients with supraglottic laryngeal tumors having a given stage lesion Neutrons only and mixed beam (6 patients)

	T1	T2	T3	T4	Total
N0	0	0	0	0	0
N1	0	0	0	0	0
N2	0	0	0	1	1
N3	0	1	1	3	5
Total	0	1	1	4	6

Table 5

mparison between the present results and the results of con itional radiation therapy for advanced lesions of the hypopharynx and supraglottic larynx

	Fraction disease free
popharynx	
sent series (flat portion of curve)	
eutrons only	0 4
lixed beam	0 4
LE & SHIGEMATSU (1976)	
adiation alone 3 year NED)	
yriform sinus (T3N0–3)	0 04
ostericoid (T3N0–3)	0
osterior pharyngeal wall (T3N0–3)	0 3
RKS et coll (1978)	
adiation and surgery 2 year NED)	
yriform sinus (T3)	0 4
(T4)	0 32
praglottic larynx	
sent series (flat portion of curve)	0 17
OSSEIN et coll (1974)	
year NED)	
T3	0 45
T4	0 49
NG (1973) (3 year NED)	
T3	0 71
T4	0 23

reated with neutrons alone 2 lesions were poorly differentiated and 3 lesions were well differentiated The primary lesions were staged according to the recommendations of the American Joint Committee for Cancer Staging and End Result Reporting which s shown in abbreviated form in Table 3 The nodal status was assessed using the same scheme as for the hypopharyngeal lesions as indicated in Table 1 The distribution of laryngeal tumors according to this staging system is shown in Table 4 As in the case of the hypopharynx it is important to note the advanced disease status of the patients This often made it difficult to determine precisely where in the larynx the tumor arose but based upon the joint clinical evaluation of the examining physicians (including the otolaryngologist who performed the endoscopic examination) all 6 lesions appeared to originate in the supraglottic region

Results

Hypopharyngeal tumors All patients who have not either died or exhibited a local recurrence at the primary site have been followed up for a minimum of 26 months Because of the difficulty in distinguishing between residual tumor and radiation induced ulceration a patient was not considered as having achieved a local control until either no possibly malignant areas were noted on examination or until biopsies of any such areas were proved to be negative If during follow up a possibly malignant area showed itself to be residual tumor then the patient was categorized as not having achieved an initial local control In a few cases the patient died before he had been adequately evaluated and if no autopsy was obtained such patients were assumed to be treatment failures All follow up times are measured from the date radiation therapy was completed

In the group of 10 patients with hypopharyngeal tumor treated with neutrons alone one was given 150 cGY$_{n\gamma}$ on a Monday–Friday basis 4 were given 100 cGy$_{n\gamma}$ on a Monday–Wednesday–Friday basis and 4 were given 75 cGy , on a Monday–Tuesday–Thursday–Friday basis One patient was treated in still another way and received 100 cGy , on a Monday–Tuesday–Thursday–Friday basis Because of holiday scheduling there were of course some modifications to these regimes In this group of patients as well as other groups with tumors of different head and neck sites (LARAMORE et coll 1979 1980) no obvious differences were found in patient tolerance to the treatment among the various neutron-only treatment regimes All patients except one completed their planned course of treatment and received between 1900 and 2200 cGy$_{\gamma}$ to the primary tumor volume and any area of massive cervical adenopathy The other patient received 1600

$cGy_{n\gamma}$ at which time a second primary was noted in a region of the cervical esophagus at the lower border of the neutron field The primary had totally regressed as had the neck node (originally staged (T3N1) and so a ^{60}Co photon field was added to the lower neck An additional 2000 cGy_{γ} was given to the primary and 5400 cGy_{γ} to the esophageal lesion using right and left posterior oblique wedge fields This patient had complete regression of both primary lesions She died 2 months later of a pulmonary infection and at autopsy she was disease free at both sites and furthermore no evidence of distant metastases was found Counting this patient the initial complete remission rate was 4/10 (40%) The subset with complete remission included 2 patients with T2 lesions and 2 patients with T3 lesions All 4 patients died free of disease at the primary sites at respective times of 2 5 6 and 10 months The patient who died at 6 months did so because of airway obstruction which was considered to be a treatment complication The patient who died at 10 months did so because of pulmonary metastases although he did have some problems with laryngeal edema 8 months after completing therapy The survival of the subset of patients who did not achieve an initial local control ranged between 2 5 and 11 months (mean 6 25 months) Of these 2 patients had an attempted surgical resection of their residual disease and lived 10 and 14 months respectively after the resections One patient was free of disease at autopsy while one had residual tumor in the neck A graphical display of the actuarially calculated (American Joint Committee for Cancer Staging and End Result Reporting 1977) fraction of patients disease free at the primary site is shown as the lower curve in Fig 1 The actuarial method was used to maximize the information obtained from a group of patients with a spectrum of follow up times

In the group of patients treated with the mixed beam regime the total radiation doses to the primary site ranged between 840 and 900 $cGy_{n\gamma}$ + 3600 to 4140 cGy_{γ} In terms of an approximate photon dose (assuming RBE = 3) the doses ranged between 6300 and 6660 cGy equivalent The initial complete remission rate for the mixed beam group of patients was 5/6 (83%) Of these 2 recurred within 2 months bringing the percentage with any significant duration of local control to about that of the group treated with neutrons alone A graphical display of the actuarially calculated (American Joint Committee for Cancer Staging and End Result Reporting) fraction of patients free of disease at the primary site is shown as the upper curve in Fig 1 The one patient (stage T3N3) who did not achieve an initial complete remission died 2 months after completing therapy when advancing tumor eroded through a carotid artery Only one patient in the subgroup who attained an initial complete remission is still alive and although he continues to be free of local and regional disease at 26 months he had developed pulmonary metastases at 22 months One other patient developed pulmonary metastases at one month and another developed hepatic metastases at 10 months A surgical salvage was attempted in one patient who recurred at 2 months but it was only partially successful Although no untoward postoperative complications occurred the patient died of progressing tumor 3 months later Excluding the one patient who is still alive the other 4 patients who achieved an initial complete remission had a mean survival of 4 5 months (range 1–10)

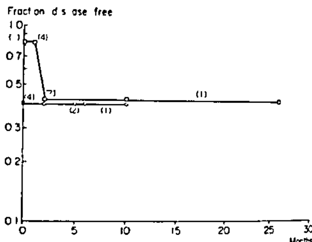


Fig 1 Fraction of patients with hypopharyngeal tumor disease free at the primary site as a function of time after completing radiation therapy The curves were calculated using the actuarial method (American Joint Committee for Cancer Staging and End Result Reporting) Ten patients treated with neutrons alone (O) 6 patients treated with a mixed beam regime (□) The ordinate is shown on a logarithmic scale and the numbers in parentheses indicate the number of patients at risk for the given data points

Both groups of patients developed the expected treatment reactions of skin erythema mucositis sore throat dysphagia decreased salivary function and altered taste sensation In the mixed beam group of patients these sequelae were about the same as would be expected from conventional photon irradiation to comparable dose levels No patient treated with the mixed beam regime required a break in treatment The treatment reactions were more severe in the group of patients treated with neutrons

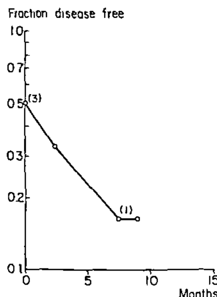


Fig 2 Fraction of patients with tumor of the supraglottic larynx disease free at the primary site as a function of time after completing radiation therapy The curve was calculated using the actuarial method (American Joint Committee for Cancer Staging and End Result Reporting) and includes patients treated both with neutrons alone and with the mixed beam regime The ordinate is shown on a logarithmic scale and the numbers in parentheses indicate the number of patients at risk for the given data points

alone Three patients required a 2 week break because of a severe moist skin desquamation and 2 patients developed significant laryngeal edema at 6 and 8 months after completing therapy All patients had an acceptable vocal quality at the time of completing radiation therapy

Supraglottic laryngeal tumors All patients have been followed up until they have either died or exhibited a local recurrence The criteria for assuming that a patient achieved an initial local control of his tumor are the same as for the group with hypopharyngeal tumors Again all follow up times are measured from the completion of radiation therapy

A total of 6 patients with advanced tumors of the supraglottic larynx were treated for cure 5 patients were treated with neutrons alone and one patient was treated with the mixed beam regime In the patients treated with neutrons alone one received 150 $cGy_{n\gamma}$ on a Monday–Wednesday–Friday basis 2 received 100 $cGy_{n\gamma}$ on a Monday–Wednesday–Friday basis and 2 received 75 $cGy_{n\gamma}$ on a Monday–Tuesday–Thursday–Friday basis Total doses to the primary site and any areas of massive cervical adenopathy ranged between 1900 and 2000 $cGy_{n\gamma}$ The patient who was treated using the mixed beam regime received 900 cGy_n + 3960 cGy_γ ~ 6660 cGy equivalent In 3 of these 6 patients (including the mixed beam patient) an initial complete remission was achieved but the average duration of this was only 6 3 months (range 2 5–9) The subset with complete remission included one patient with a T2 lesion one patient with a T3 lesion and one patient with a T4 lesion In this subset one patient died free of disease at 9 months and one patient exhibited a local recurrence at 2 5 months and underwent a surgical salvage procedure This patient had received 1900 $cGy_{n\gamma}$ to the primary and involved cervical neck nodes (initial stage T3N3) and severe postoperative wound healing problems occurred Although this patient lived an additional 11 months after the initial surgery repeated surgical manipulations were required to remove nonviable tissue and death was ultimately due to erosion through a carotid artery This is in contrast with the patient receiving the mixed beam irradiation (initial stage T2N3) who underwent a salvage surgical procedure when he recurred at 7 5 months without showing any postoperative wound healing complications This latter patient is still alive and doing well 42 months after the surgery The 3 patients who did not achieve an initial complete remission had a mean survival of only 3 3 months (range 2–6) A graphical display of the actuarially calculated (American Joint Committee for Cancer Staging and End Result Reporting) fraction of patients disease free at the primary site appears in Fig 2

The complications of radiation therapy were essentially the same as listed for the group of patients with hypopharyngeal tumors In the group of patients treated with neutrons alone one developed a marked subcutaneous edema shortly after completing treatment and another patient developed a moist desquamation severe enough to warrant a one week break in the treatment The patient treated with the mixed beam regime had no untoward difficulty All patients had an acceptable vocal quality after completing radiation therapy

Discussion

The results of a Phase I preliminary trial carried out at this University were described in which fast neutrons from a cyclotron designed for physics research were used to treat patients with advanced tumors of the hypopharynx and supraglottic larynx Because this was a Phase I trial designed as much to investigate the toxicity of neutron radiation as to

gain clinical information about its tumoricidal potential only patients with advanced lesions who were considered to have less than a 10 per cent 5 year survival with conventional treatment modalities were accepted The short survival times of the patients who did not show an initial complete response to therapy bear this out In the case of patients with hypopharyngeal tumor it was possible to discuss separately the subgroups treated with neutrons alone and with a mixed beam regime Although the numbers in the 2 groups are small the results support other reports (GRIFFIN et coll 1978 1979 LARAMORE et coll 1979 1980) which indicate that the mixed beam regime is at least as effective as neutrons alone and that the side effects both alone and in connection with subsequent surgery (GRIFFIN et coll 1979) are no more severe than those associated with photon radiation alone This is important since it provides a way of maximally utilizing treatment time on neutron therapy machines and this is likely to be fairly limited in the foreseeable future The side effects of the neutron-only treatment regimes were more severe than the mixed beam regime and a high complication rate occurred in patients when a surgical salvage was attempted after full dose radiation However even when conventional photon radiation is used in a preoperative manner VANDENBROUCK et coll (1977) found a high percentage of postsurgical complications for hypopharyngeal tumors

In the group of patients with hypopharyngeal tumor the local control rate as shown in Fig 1 plateaued at about 0 4 for both the neutron and the mixed beam group However only one patient was at risk beyond 10 months Four of the 9 patients with initial local control developed distant metastases which accounts in part for the poor survival in spite of there being initial local control The tendency of advanced lesions involving the pyriform sinuses to develop distant metastases has been previously documented (MARKS et coll) and the present results tend to bear this out This is particularly the case when the patients have advanced cervical adenopathy as well A crude comparison between the plateau phase figures and the results quoted in the literature for the local control rate of advanced hypopharyngeal tumor is done in Table 5 INOUE & SHIGEMATSU break their results down according to site of origin and find considerably better results for posterior pharyngeal wall tumors than for tumors arising in either the pyriform sinus or the post cricoid regions These figures come from their Tabl 2 excluding the long term survivors due to salvag surgery MARKS et coll quote figures for planne preoperative radiation followed by surgery at ~ 0 3 for T4 lesions of the pyriform sinus Although the follow up time of 2 years is considerably longer tha the present ones their figures are comparable wit the plateau phase figures obtained with radiatio alone

A similar comparison has been made in Table for the group of patients with supraglottic tumors At 2 years GHOSSEIN et coll (1974) reported NED survivals of 0 45 and 0 49 for T3 and T4 lesions respectively WANG reported corresponding results at 3 years of 0 21 for T3 lesions and 0 23 for T4 lesions although it should be noted that 49 per cent o his patients were initially staged N0 and all of the present patients were stage N2-N3

The advanced group of tumors in the present series makes comparison with the literature results quite difficult The current investigation on the use of high LET neutron radiation for squamous cel carcinoma of the head and neck area is a *randomized* prospective trial which directly compares this with conventional photon radiation Because of the morbidity of definitive neutron radiation alone shown in the early Phase I trial this institution and most of the other neutron institutions in the United States are using only a mixed beam treatment regime for the high LET arm Preliminary results indicate that this is more effective at local control than the *photon arm but it is much too early* to say anything about improved survival

Conclusion

The analysis of a Phase I clinical trial testing the efficacy of fast neutron radiation therapy for ad vanced carcinoma of the hypopharynx and supra glottic larynx reveals local control rates comparable with photon radiation therapy of lesions which were probably less advanced The control rate using a mixed beam (neutron/photon) treatment regime seems to be as good as that achieved with neutron radiation alone but with less associated adverse effects

SUMMARY

The results of a Phase I clinical trial using fast neutron teletherapy for advanced squamous cell carcinoma of the hypopharynx and supraglottic larynx are reported Ten patients with hypopharyngeal tumors were treated for cure using neutrons alone and 6 patients with hypophar

eal tumors were treated for cure using a combination neutrons and photons as part of a mixed beam frac tation scheme In the neutron only group the initial mplete remission rate was 4/10 (40%) The mean sur al of this group of patients with initial complete remis i was only 5 75 months (range 2–10) but all patients l without evidence of recurrent tumor at the primary In the mixed beam group the initial complete remis i rate was 5/6 (83%) but the local control rate dropped dly to 41 per cent when displayed on an actuarial plot patients with advanced tumors of the supraglottic nx were treated for cure 5 with neutrons alone and with a mixed beam regime The initial complete remis i rate was 3/6 (50%) but 2 of these patients rapidly rred which reduced the local control rate on an actu l plot to 16 per cent The relative adverse effects of neutron-only treatment regime appear to be substan) greater than those of the mixed beam treatment me

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OM THE DEPARTMENT OF GENERAL ONCOLOGY RADIUMHEMMET KAROLINSKA SJUKHUSET S-104 01 STOCKHOLM SWEDEN

YPOTHYROIDISM FOLLOWING 131I THERAPY FOR HYPERTHYROIDISM IN RELATION TO IMMUNOLOGIC PARAMETERS

G LUNDELL and L E HOLM

munologic reactions are known to be of impor : in the development of hypothyroidism after ment for hyperthyroidism Postoperative hypo oidism is more frequent when the surgical oid specimens reveal marked lymphoid infiltra (WHITESELL JR & BLACK 1949 HARGREAVES ARNER 1968) A higher incidence of postopera hypothyroidism was found in patients who be treatment had demonstrable antibodies to oid antigens (HJORT & MOGENSEN 1962) 131I ment for hyperthyroidism is followed by hypo oidism in a considerable proportion of the cases SSON 1955 WERNER et coll 1957 BELING & IORN 1961 BECKER et coll 1971) A correla between thyroid autoantibodies after 131I treat t and hypothyroidism developing early after the apy has been found (BLAGG 1960 SKANSE & SSON 1961 EINHORN et coll 1965) as well as rrelation between the presence of thyroid anti es before 131I therapy and the development of othyroidism following such therapy (LUNDELL ONSSON 1973) Whether this correlation found by IDELL & JONSSON still existed after a longer ow up period of the same patient group has now n investigated

Material and Methods

The original series (LUNDELL & JONSSON) com sed 188 patients (144 females 44 males) with con ned hyperthyroidism and in whom sera for determination of antibodies to thyroid antigens had been collected before and after 131I therapy during the period 1963 to 1969 A further 3 patients also treated at this hospital during the same period and where sera had been collected in the same manner could be added to the group of 188 patients The median age of the 191 patients was 55 years (range 23–83 years)

Fourteen per cent of the patients (n=27) had no clinically demonstrable goiter 46 per cent (n=88) a diffuse goiter and 40 per cent (n=76) a nodular goiter The goiters were assessed on the basis of palpation as being moderately or markedly enlarged None of the patients had previously received a therapeutic dose of 131I but 37 patients had been operated upon

The principles of 131I therapy have been reported elsewhere (LARSSON BELING & EINHORN) The amount of 131I administered at each treatment varied between 74 MBq (2 mCi) for patients without demonstrable goiter and 1 295 MBq (35 mCi) for patients with large nodular goiters and the dose was calculated without knowledge of the thyroid antibody titers When the initial 131I dose was insufficient to control the hyperthyroidism further doses were given usually at a 3 month interval Sixty-one per cent of the patients received one dose 25 per cent two doses 8 per cent three doses and 3 per cent of the patients received between five and seven

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doses The mean initial dose administered was 270 MBq (7 3 mCi) and the mean total dose 470 MBq (12 7 mCi range 74–3 700 MBq)

The patients were examined regularly after treatment at intervals of 2 to 4 months during the first year and subsequently at longer invervals usually 6 months to one year until they developed hypothyroidism or up till 31 December 1978 For patients who received more than one ¹³¹I treatment the follow up period always dated from the time of the initial treatment Classified as hypothyroid were all patients requiring permanent thyroid replacement therapy The diagnosis of hypothyroidism was based on the clinical condition of the patient supported by laboratory analyses of sera and radioiodine tracer tests If permitted on clinical grounds thyroid replacement therapy was generally avoided during the first 4 months after the ¹³¹I therapy since transient hypothyroidism may occur within the first months of such therapy

Sera were collected from each patient before therapy and at intervals thereafter during the first year afterwards Antibodies to thyroid cytoplasmic antigen were determined by Coons direct immunofluorescence technique (ROITT & DONIACH 1958 HOLBOROW et coll 1959) Antibodies to thyroglobulin were determined by the Boyden passive hemagglutination test with tanned red cells (DERRIEN et coll 1948 BOYDEN 1951 WITEBSKY & ROSE 1956) Patients with titers of less than 1/20 of antibodies to thyroglobulin were classified as negative and those with titers of 1/20 or more as positive

The cumulative incidence of hypothyroidism was calculated using standard life table techniques The statistical methods used were the log rank test and the Student s t test

Results

The cumulative incidence of hypothyroidism after ¹³¹I treatment for hyperthyroidism is shown in Fig 1 After one year 25 per cent of the patients without demonstrable goiter 13 per cent of those with diffuse goiter and 5 per cent of the patients with nodular goiter were hypothyroid At the end of the follow up the cumulative incidence of hypothyroidism was 63 per cent for patients without goiter 57 per cent for those with diffuse goiter and 36 per cent for patients with nodular goiter The differences were statistically significant (p<0 01) between the group with nodular goiter and each of the two other groups but not between the group with diffuse goiter and that without goiter

The incidence of hypothyroidism in the 37 patients operated upon before ¹³¹I treatment was similar to that of the other 154 patients

Of the 75 patients who before treatment had demonstrable antibodies to thyroid cytoplasmic antigen 20 per cent were hypothyroid within one year and 66 per cent within 12 years of the treatment as compared with somewhat lower figures for those with demonstrable antibodies to thyroglobulin (n=88 Fig 2) Patients who lacked both antibodies (n=81) exhibited a lower cumulative incidence than

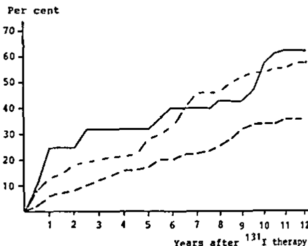


Fig 1 Cumulative incidence of hypothyroidism after ¹³¹I therapy for hyperthyroidism — No demonstrable goiter (n=?7) - - Diffuse goiter (n=88) - - Nodular goiter (n=76)

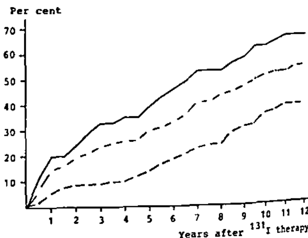
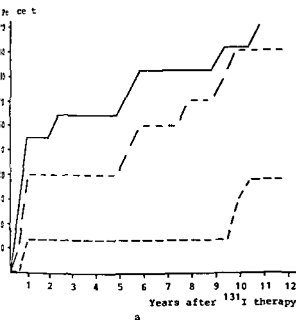
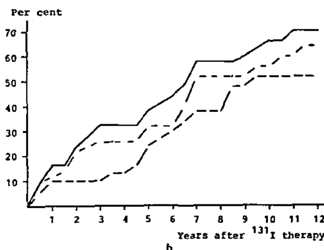


Fig 2 Cumulative incidence of hypothyroidism after ¹³¹I therapy in patients with or without thyroid antibodies before therapy Patients with antibodies to both cytoplasmic antigen and thyroglobulin are included in both seropositive groups — With antibodies to cytoplasmic antigen - - With antibodies to thyroglobulin - - Without antibodies





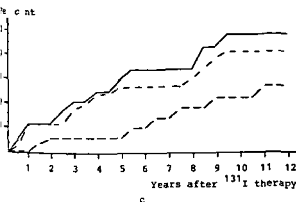


Fig 3 Cumulative incidence of hypothyroidism after ¹³¹I therapy in patients with a) no goiter b) diffuse goiter c) nodular goiter Patients with antibodies to both cytoplasmic antigen and thyroglobulin are included in both seropositive groups — With antibodies to cytoplasmic antigen - - With antibodies to thyroglobulin — — Without antibodies

tients with antibodies to cytoplasmic antigen or yroglobulin or both ($p<0\ 05$) 6 per cent within ne year as compared with 39 per cent within 12 ears of therapy

Fig 3 demonstrates the significance of the presence of thyroid antibodies before treatment in the development of hypothyroidism in relation to the type of goiter Six of the 11 patients without demonstrable goiter (Fig 3 a) and who displayed antibodies to thyroid cytoplasmic antigen became hypothyroid within one year and all the cases were hypothyroid within 12 years of the treatment Patients with demonstrable antibodies to thyroglobulin (n=10) had an incidence of hypothyroidism that was similar to that of patients with demonstrable antibodies to thyroid cytoplasmic antigen although somewhat delayed In those patients without detectable antibodies before treatment (n=14) 14 per cent were hypothyroid within one year and 38 per cent within 12 years of treatment The difference between the seropositive (cytoplasmic antigen or thyroglobulin or both) and seronegative groups was statistically significant ($p<0\ 01$)

In the group of patients with diffuse goiter (Fig 3 b) and presence of antibodies to thyroid cytoplasmic antigen before therapy 14 per cent of the cases were hypothyroid within one year and 63 per cent within 12 years Patients who before treatment had antibodies to thyroglobulin had a similar but somewhat lower cumulative incidence of hypothyroidism Thirty three patients had no detectable antibodies before treatment and they had a slightly lower cumulative incidence than the seropositive patients However the differences between the three curves were not statistically significant

Seventy-six patients had a nodular goiter (Fig 3 c) and the cumulative incidence of hypothyroidism was lower for patients without demonstrable antibodies

before therapy (n=34) but the differences were not statistically significant

Fig 4 shows the cumulative incidence of hypothyroidism in patients with demonstrable antibodies to thyroid cytoplasmic antigen or thyroglobulin or both before therapy in relation to the type of goiter It seems evident that the presence of these antibodies sooner or later results in hypothyroidism in almost all patients without goiter The group of diffuse goiter had a similar progressive hypothyroidism although at a slower rate A longer follow up period is needed to ascertain whether the group with nodular goiter reaches a plateau as is suggested in the figure

Of the 119 patients without demonstrable antibodies to thyroid cytoplasmic antigen before the ^{131}I therapy (Fig 5) 52 developed such antibodies afterwards while 67 remained seronegative with respect to cytoplasmic antigen titers for at least one year A significantly higher incidence of hypothyroidism was found in the group becoming seropositive ($p<0.01$) Of 104 patients without demonstrable antibodies to thyroglobulin before therapy 33 developed such antibodies within one year No correlation was found between the incidence of hypothyroidism and the presence of antibodies to thyroglobulin developing after the therapy in comparison with those remaining seronegative for that antibody Patients with diffuse goiter or not palpable thyroid glands more often developed antibodies to thyroid cytoplasmic antigen following therapy than patients with nodular goiter

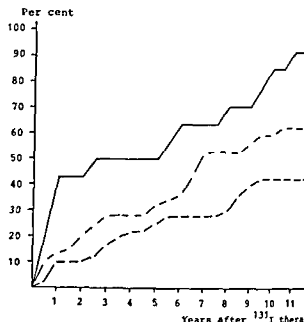


Fig 4 Cumulative incidence of hypothyroidism after ^{131}I thera in patients with thyroid antibodies to cytoplasmic antigen thyroglobulin or both before therapy — No goiter - - Diffu goiter - - Nodular goiter

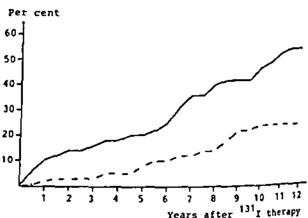


Fig 5 Cumulative incidence of hypothyroidism after I therapy in patients without antibodies to cytoplasmic antigen before therapy — Seropositive to cytoplasmic antigen after therapy - - Seronegative to cytoplasmic antigen after therapy

Discussion

^{131}I therapy for hyperthyroidism is followed by hypothyroidism in a considerable number of patients Between 7 and 20 per cent of the patients become hypothyroid during the first year after therapy and the cumulative incidence increases to 25 to 50 per cent within 7 to 12 years of treatment (BELING & EINHORN) At present all patients treated with ^{131}I for hyperthyroidism between 1951 and 1977 at Radiumhemmet are analysed The results after the analysis of the first 3 000 of these cases show that the cumulative incidence of hypothyroidism increases steadily to 70 per cent within 25 years after such therapy

^{131}I therapy for hyperthyroidism may trigger an autoimmune reaction manifested as an increase in humoral thyroid antibodies (EINHORN et coll 1965 O GORMAN et coll 1964) This also occurred after ^{131}I therapy in euthyroid subjects (EINHORN et coll 1966) Previously it was shown that patients who had demonstrable antibodies before ^{131}I treatment developed hypothyroidism to a greater extent than those without such antibodies (LUNDELL & JONSSON) The present results show that this difference persists as long as 12 years after treatment In the previous series hypothyroidism developing within one year after the ^{131}I therapy also occurred significantly more often in patients without demonstrable antibodies to thyroid cytoplasmic antigen before

atment but who developed such antibodies after atment than in those who remained seronegative LNDELL & JONSSON) With the prolonged ob nation period of the present series a significant ference was found also for hypothyroidism devel ing more than one year after the therapy

The increase in thyroid antibodies after 131I thera is temporary and lasts about one year and at time of hypothyroidism the level of humoral ibodies usually is at pre treatment or lower levels INHORN et coll 1965 1966) It is thus not likely it this humoral autoimmune reaction caused by 131I therapy can alone account for the continued rease in the cumulative incidence of hypothyroi m many years after treatment It is not known ether 131I therapy also triggers an autoimmune l mediated response If so such a response could ntribute to the progressive development of hypo roidism Another explanation could be a progres e atrophy similar to that which has been observed er external irradiation (EINHORN et coll 1965)

The present analysis shows that the results pre usly reported at an early stage of the same group patients (LUNDELL & JONSSON) are valid also for onger follow up period It is evident that thyroid ibodies—and mainly those to thyroid cyto smic antigen—as well as the type of goiter are importance in the development of hypothyroidism er 131I therapy for hyperthyroidism Immunologic tors must therefore be taken into account when nparing the results of different 131I treatment edules for hyperthyroidism

The level of thyroid antibodies before treatment ıld be a valuable factor when determining the e of 131I administered for the treatment of hyper roidism Patients with a high risk of developing pothyroidism could possibly be treated with her doses thereby curing them without unneces ry delay but also bringing about the onset of pothyroidism more rapidly Some of the problems ising in the follow up of these patients would then diminished

SUMMARY

A series of 191 patients with hyperthyroidism treated ith 131I was examined for the presence of antibodies to yroid cytoplasmic antigen and to thyroglobulin before nd after therapy and followed up for 12 years Patients ith thyroid antibodies before therapy had a significantly igher incidence of hypothyroidism than those without demonstrable antibodies ($p<0.01$) A higher incidence of hypothyroidism occurred in the presence of antibodies to thyroid cytoplasmic antigen than to thyroglobulin Patients without demonstrable antibodies to thyroid cyto plasmic antigen before the therapy and who afterwards developed such antibodies had a significantly higher incidence of hypothyroidism than those who remained seronegative ($p<0.01$)

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FROM THE DEPARTMENT OF GENERAL ONCOLOGY RADIUMHEMMET KAROLINSKA SJUKHUSET S-10401 STOCKHOLM SWEDEN

THYROID TREATMENT AND ITS POSSIBLE INFLUENCE ON OCCURRENCE OF MALIGNANT TUMORS AFTER DIAGNOSTIC ^{131}I

L E HOLM

Previously HOLM et coll (1980 a b) found that the number of malignant thyroid tumors in a population of 10133 patients who had received diagnostic doses of ^{131}I for possible malignant thyroid tumor or for thyroid dysfunction did not differ from the number expected from the Swedish Cancer Registry data When deaths were accounted for the mean follow up period was 17 years Nine of these patients were reported to the Registry as having a malignant thyroid tumor against an expected figure of 8 3 On the other hand a large difference was found between the number observed and the number expected on the basis of the risk estimates for radiation induced malignant thyroid tumors given by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR 1977) when these risk estimates were used after correction for the actual size of the thyroid glands the computed number of such tumors was 47–124 (HOLM et coll 1980 a) The observed incidence of malignant thyroid tumors could have been affected by thyroid surgery thyroid hormone medication or other forms of thyroid treatment and the purpose of the present report is to present the extent to which such therapy was given after the administration of the diagnostic doses of ^{131}I

Material and Methods

Between 1952 and 1965 diagnostic doses of on average 2 22 MBq (60 μCi)—0 11 to 34 8 MBq (3–940 μCi)—were given at Radiumhemmet to 8047 female and 2086 male patients for possible malignant thyroid tumor or thyroid dysfunction The Swedish Cancer Registry records for 1958–77 were searched for the occurrence of malignant thyroid tumors in any of the 10133 patients In this follow up a latency period of 5 years was applied thereby excluding the first 5 person years for each patient and also tumors appearing in the Registry within 5 years of the first ^{131}I examination (HOLM et coll 1980 b)

In a sample of this population comprising all those patients born on days 5 15 and 25 of each month the extent was analysed to which thyroid therapy was given during the follow up time which was taken as the period from the first administration of ^{131}I to 31 December 1977 or until death The statistical sample numbered 968 patients (770 females and 198 males) This group did not differ from the whole patient population as regards either the mean age (44 years) or the mean activity of ^{131}I measured in the thyroid gland 24 hours after the administration of the nuclide (0 89 MBq)

The addresses of those patients still alive in August 1979 were obtained from the parish records Patients who had died were divided into two groups according to whether they had survived for up to 5 years or for a longer period after the ^{131}I examination

Since the latency period of 5 years had been applied in the previous calculations (HOLM et coll

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1980 b) the 97 patients dying within 5 years were not included in the present series Of the remaining 871 patients of the statistical sample 187 had died by August 1979 9 had emigrated and 10 could not be traced The patients who had emigrated or who could not be traced were assumed to be alive on August 1979 and the thyroid treatment was classed as not known

A questionnaire concerning any therapy for thyroid diseases was mailed to all 665 patients who were still alive in August 1979 and who could be located It was accompanied by a letter explaining that an investigation was being carried out to ascertain the reliability of the diagnostic ^{131}I examination and its usefulness in medical practice The patients were told that their answers would be helpful in deciding whether and if so how this examination could be improved (The information so gathered will be analysed in a coming report) No mention was made of the previous report on the incidence of malignant thyroid tumors following diagnostic doses of ^{131}I

The patients were asked whether they had ever had thyroid surgery or received radioiodine therapy for any reason In cases where such treatment had been given the name of the hospital and the approximate year of the treatment were requested Patients that had received thyroid hormones or antithyroid drug therapy were requested to give the year when it was introduced Those that had taken thyroid hormones as the sole form of treatment (n=102) were asked by telephone for how many years they had continued the medication For 7 of these pateints that could not be reached it was assumed that the drug had been taken continuously until the end of the follow up period on 31 December 1977

Of the 665 patients receiving the questionnaire 628 answered and a further 22 could be reached by telephone In 12 of the remaining 15 patients the records at the referring hospital yielded the required information Thus in only 3 of the 665 patients were such data not obtained

For the 187 patients who survived more than 5 years but had died by August 1979 every effort was made to gather information as to thyroid surgery thyroid hormone medication or other forms of thyroid treatment received after the ^{131}I examination by searching their records at the referring hospitals at the hospitals where they had died or at any other relevant hospital mentioned in the records In 133 of these 187 patients the information was deeme adequate and reliable In the remaining 54 patient the thyroid treatment was classed as not known

Information on any form of thyroid treatment re ceived after the ^{131}I examination was thus obtaine for 795 of the 871 patients (91%) surviving mor than 5 years

The types of thyroid operation were classified a hemithyroidectomy or partial resection of on thyroid lobe bilateral subtotal thyroidectomy an not known

The data were recorded on a PDP 11/35 computer using an interactive input program The analysi programs were run on the same computer

Results

Of the 871 patients of the statistical sample wh were still living 5 years after the first ^{131}I examina tion 507 (58%) received no form of thyroid treat ment during the follow up period Inadequate infor mation was more common among the patients tha died than among the survivors (Table 1)

Thyroid treatment of some kind was receive within one year of the examination by one quarter o the 871 patients and some time during the follow up period by one third (Table 2) Surgery accounted fo 69 per cent of the treatments given within the first year and for 58 per cent over the whole period The most common surgical procedure was bilateral sub total thyroidectomy (77%) followed by partial and hemithyroidectomy (10%) the remaining 13 per cent were classed as not known No total thyroidec tomy was performed on any patient About one third of the patients undergoing surgery did not re ceive thyroid hormone therapy post operatively

The second most common form of treatment was thyroid hormone medication that accounted for 27 per cent of the treatments given within the first year and for 35 per cent over the whole period For the 102 patients given this as the sole form of treatment the mean period of medication was 10 years which was about two thirds of the follow up period for these patients In 76 patients no information was available

The distribution of the patients receiving thyroid surgery or hormone treatment according to the reason for performing the ^{131}I examination appears in Table 3

Table 1

Distribution (per cent) of the statistical sample

Treatment	Patients		
	Alive (n=684)	Dead (n=187)	Total (n=871)
No thyroid treatment	62	44	58
Thyroid treatment	35	27	33
Not known	3	29	9

Table 2

Patients of the statistical sample surviving more than 5 years after the ¹³¹I examination distributed by the type of treatment

Type of treatment	Treatment given within 1 year of ¹³¹I examination		Treatment given during follow up ending 31 December 1977	
	No	Per cent	No	Per cent
Surgery with or without thyroid hormone supplement therapy	147	17 0	168	19 4
Thyroid hormones only	57	6 5	102	11 7
Thyroid hormones + antithyroid drugs	3	0 3	8	0 9
Antithyroid drugs	6	0 7	7	0 8
¹³¹I therapy	0	0	3	0 3
No treatment	582	66 8	507	58 2
Not known	76	8 7	76	8 7

Table 3

Patients undergoing thyroid surgery or given thyroid hormone treatment distributed by reasons for performing the ¹³¹I examination

Reason	Total No of patients	Surgery (per cent)	Thyroid hormone (per cent)
Possible malignant thyroid tumor	351	33	12
Hyperthyroidism	390	12	5
Hypothyroidism	152	1	20
Other reasons	75	4	13

Discussion

The extent to which thyroid surgery and thyroid hormone medication may have affected the incidence of malignant thyroid tumors in the present patient population is difficult to establish Surgery might be considered to reduce the probability of a tumor developing because of the removal of thyroid tissue But it might also be argued that where surgery is not followed by thyroid hormone therapy the risk of a malignant tumor developing could be even greater than when no operation is performed because of the possible increase in the thyroid stimulating hormone level

Thyroid hormones were administered to 12 per cent of the patients of the statistical sample There is evidence that the growth of papillary and possibly follicular carcinomas can be arrested for some time by the administration of thyroid hormones (WERNER 1971) The risk of developing a radiation induced malignant tumor might have been reduced by the thyroid hormone therapy (DONIACH 1977) However the efficacy of thyroid hormones in preventing thyroid tumor formation in humans many years after radiation exposure is still unknown

Among the whole population of the 10 133 patients given diagnostic doses of ¹³¹I 9 were reported to the Swedish Cancer Registry as having a malignant thyroid tumor In 4 of these 9 patients thyroid surgery had been performed some time after the diagnostic examination and before the tumor diagnosis 2 of them had received thyroid hormones postoperatively In these 4 patients neither the thyroid surgery nor the administration of thyroid hormones was apparently effective in preventing development of the malignant tumor

Three patients of the statistical sample had received radioiodine therapy for hyperthyroidism The reason for this low number is that radioiodine therapy in this region of Sweden was at that time given almost exclusively at Radiumhemmet and all patients known to have received such therapy as well as external radiation therapy were excluded from the series (HOLM et coll 1980 b) The intervals between the diagnostic and therapeutic ¹³¹I doses for the 3 patients of the sample were 11 13 and 14 years respectively and in none of them was hyperthyroidism the reason for performing the initial diagnostic examination

Radioiodine therapy seems not to be associated with an increased incidence of malignant thyroid tumors (HOLM et coll 1980 c) but perhaps even reduces the probability of such tumors developing by virtue of the high doses of ¹³¹I administered (Committee on the Biological Effects of Ionizing Radiations 1972 UNSCEAR 1977)

Most of the 76 patients on whom no information concerning thyroid treatment could be obtained were among those that died Because the informa tion on these patients was sought only at hospitals it is possible that this group includes patients who did not receive thyroid treatment and where adequate information could therefore not be obtained Support for this possibility is found in the fact that the proportion of patients with no thyroid treatment was lower for the patients who died

Because the determination of the effect of the various forms of thyroid treatment on the occur rence of radiation induced malignant thyroid tumors is unreliable the fact that at least 58 per cent of the statistical sample did not receive any thyroid therapy during the time they were at risk is an im portant finding In addition among the 9 patients who were reported to the Swedish Cancer Registry because of a malignant thyroid tumor 4 had had thyroid surgery and 2 had received thyroid hor mones postoperatively In view of the long follow up period the difference between the number of malignant thyroid tumors observed in the 10 133 pa tients who had received diagnostic doses of ^{131}I and the number computed from UNSCEAR s risk fig ures is not ascribable to the thyroid treatment to more than possibly a small extent

A conceivable explanation for this difference is the much lower dose rate of ^{131}I than of external irradiation on which UNSCEAR s risk figures are mainly based Furthermore it is not known whether a radiation dose threshold for the induction of malignancy in humans exists although the non linear relationship between dose and effect implies that there is little if any risk at low dose levels in cases of cataract of the lens or impairment of fertili ty

Animal experiments often indicate that the dose–response curve varies according to the types of Linear Energy Transfer (LET) the curve being sig moid for low LET and linear for high LET ISHIMARU et coll (1971 1979) found that the incidence of leukemia in humans after exposure to the radia tion from atomic bombs was increased at doses of less than 1 Gy in Hiroshima but not in Nagasaki the discrepancy might be due to differences in the types of radiation delivered—neutrons and gamma in Hiroshima and mainly gamma in Nagasaki

The fact that also other authors have not disclosed any increased incidence of leukemia or malignant tumors at low doses of low LET radiation suggests that the dose–response curve may not conform to the linear non threshold hypothesis (RALLISON et coll 1974 FRIGERIO & STOWE 1976 SAKKA 1978)

In this series of 10 133 selected patients exposed to radiation from from ^{131}I with a mean dose of 1 59 Gy for those less than 20 years of age and 0 58 Gy for those older than this at the time of the diagnostic examination (HOLM et coll 1980a) no increased incidence of radiation induced malignant thyroid tumors was disclosed during the present follow up period It would seem that the UNSCEAR risk fig ures for radiation induced malignant thyroid tumors are not applicable to the radiation emitted by ^{131}I A longer follow up of this patient population is needed to elucidate if the results are confirmed over a longer observation period

SUMMARY

The extent to which thyroid surgery thyroid hormone medication or other forms of thyroid therapy were applied after diagnostic administration of ^{131}I has been determined in 871 patients of the 10 133 patients reported previously *The relevant information was obtained from* question naires and from hospital records Of the 871 patients at least 58 per cent had received no form of thyroid treatment and 33 per cent had had some form of treatment while in 9 per cent no information could be obtained The large dif ference between the number of malignant tumors ob served in the 10 133 patients after exposure to ^{131}I and the number expected from UNSCEAR s risk figures for radia tion induced thyroid carcinoma (47–124 cases) cannot be ascribed to the various forms of thyroid treatment given after the ^{131}I administration

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BONE ABNORMALITIES IN PATIENTS WITH MEDULLARY CARCINOMA OF THE THYROID

B RASMUSSON

Medullary carcinoma of the thyroid (MCT) con ıtıtutes about 10 per cent of all carcinomas of the hyroid It differs from the other thyroid carcinomas n several ways the most important being secretion of pathologically high amounts of the serum-calcium owering hormone calcitonin which probably is ecreted during several years before the thyroid arcinoma becomes clinically recognizable as a alpable tumor in the thyroid

MCT occurs in three apparently unrelated forms ither as a part of multiple endocrine neoplasia ype 2a (MEN 2a) which incorporates MCT arathyroid adenoma and pheochromocytoma or nultiple endocrine neoplasia type 2b (MEN 2b) hich consists of MCT pheochromocytoma pe ipheral mucosal neuromas with or without mar anoid body build and sporadic cases

The present investigation was carried out in order o evaluate the frequency and appearance of bone bnormalities in patients with all three forms of ICT

Material and Methods

All of 28 patients with microscopically proven medullary thyroid carcinoma treated and controlled in this hospital in the period 1962 through 1978 were screened for bone abnormalities by clinical examina tions and conventional radiography as well as by records also from other hospitals Twenty-one patients had serum calcitonin measured Additional examinations and measurements were performed in 5 patients photon beam absorption of the radius and the heel 25 hydroxy calciferol immunoreactive parathyroid hormone and calcium in serum as well as hydroxyproline in the urine Immunoreactive calcitonin was measured by the method described by ALMQVIST et coll (1974) 25 hydroxy calciferol was measured according to the method described by LUND & SØRENSEN (1979) Parathyroid hormone (PTH) was measured by radioimmunoassay with a double antibody technique the antibody being directed against the c terminal end of the PTH molecule (RASMUSSON et coll 1978)

The frequencies and appearances of bone affec tions were compared with those of 104 patients with other thyroid carcinomas i e papillary follicular and anaplastic carcinomas treated in this hospital in the same period

Results

Fourteen of the 28 patients with MCT had at least one bone abnormality (Table 1) All of the 6 patients who had the diagnosis of medullary thyroid carcinoma established before 32 years of age had bone abnormalities Epiphyseal slip in the proximal femur occurred in 3 patients when they were 12 to 17 years old

Schmorl nodes were present in 4 patients for more than 5 years without any herniation of the nucleus pulposus

Bone cysts unaltered during at least 5 years appeared in 3 patients the localizations were in the clavicle the acetabulum head of femur and the sacrum

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Table 1

Appearance of bone abnormalities in patients with medullary thyroid carcinoma

Age sex	Marfanoid body build*	Dolichocephaly	Prognathism	Ant chest deformity	Scoliosis	Kyphoscoliosis	Spina bifida *	Slipped epiphysis	Bone cysts	Schmorl nodes	Pes cavus	Remarks
16 M	+	+	+	+	+		+	+		+	+	MEN 2b
17 M	+	+	+			+		+			+	MEN 2b
13 M	+	+	+		+		+				+	MEN 2b
30 F	+								+			
21 M										+		
31 M	+	+	+			+						MEN 2b
38 F	+				+			+	+	+	+	
59 F					+				+			Recklinghausen s disease
60 M					+							
59 F					+		+					
67 M			+							+		
43 M					+							
59 F					+							
67 M	+			+		+					+	

* Further 3 patients had marfanoid body build but otherwise no bone abnormalities
* Two S1 one C5–C6

Three patients had spina bifida (2 in S1 one in C5–C6) 2 of them had alpha$_1$ fetoprotein measured in serum it was normal in both

The mineral content of the extremities was measured in 5 patients with high serum calcitonin and was compared with that of 5 sex and age matched control patients with papillary or follicular thyroid carcinomas and normal serum calcitonin The serum values of PTH calcium and 25 hydroxy calciferol were within normal range in both groups except in one patient with MCT who had elevated 25 hydroxy calciferol because of D vitamin intake before the blood collection The mineral content was slightly increased in one patient with MCT in the control group 3 of the patients had increased values

Biopsy from the iliac crest was performed in 2 patients In one an increased amount of osteoid was found in the other the biopsy was normal

Four patients developed bone metastases one patient osteolytic another patient osteosclerotic In the remaining 2 patients the type of metastases could not be diagnosed because they were found at a ^{131}I scintigraphy and at autopsy respectively

When compared with the bone abnormalities found in patients with other thyroid carcinomas the frequency of scoliosis was equal (Table 2) In all 34 of 104 patients with other thyroid carcinomas had bone abnormalities practically all of them had scoliosis

Three illustrative cases are described

Case 1 A 16-year-old male was operated upon for a medullary thyroid carcinoma in 1962 Two years previously he was operated upon for a slipped femoral epiphysis and it was then noted that he had kyphoscoliosis bilateral pes cavus and highly arched palate In 1975 a second operation for recurrent MCT was performed At this time it was noted that he had a marfanoid build loose jointedness hypertrophied gums with the teeth widely spaced as well as multiple mucosal neuromas located in the eyelids the lips the tongue and the larynx Serum calcitonin was extremely high >30 000 pmol/l (normal range 120–290 pmol/l) PTH and serum calcium were normal and so was the urinary excretion of hydroxyproline The mineral content was slightly increased to 41 and 32 units in the heel and the radius normal values being 34±5 and 26±4 respectively A biopsy from the iliac crest revealed increased amount of osteoid 8 3 per cent normal spongiosa and normal surface resorption

The combination of MCT skin mucosal neuromas and marfanoid body build implies that this patient belongs to the group of MEN 2b he had or has however no indication of pheochromocytoma

Table 2

The types of bone abnormalities in 14 patients with medullary carcinoma of the thyroid (MCT) and in 34 patients with other thyroid carcinoma

Bone abnormality	MCT	Other thyroid carcinoma
Dolichocephaly	4	
Prognathism	5	
Funnel chest	1	
Carinate chest	1	
Scoliosis	8	30
Kyphoscoliosis	3	3
Spina bifida	3	1
Slipped femoral epiphysis	3	
Bone cysts	3	
Schmorl nodes	4	
Pes cavus	5	

Two S1 one C5–C6

Case 2 The diagnosis of MCT was made when the patient was 39 year old At 14 years she was operated upon for left and right proximal femoral slipped epiphyses A year previously she was operated upon for MCT clinical examinations and radiography revealed bilateral pes cavus scoliosis a few bone cysts scattered in both femora and acetabulum as well as multiple Schmorl nodes Three years later recurrence of the thyroid carcinoma developed and serum calcitonin rose to >140 000 pmol/l PTH calcium and 25 hydroxy calciferol in serum were normal and so was the bone mineral content No mucosal neuromas and no signs of pheochromocytoma were found The patient thus had MCT in its monosymptomatic form

Case 3 As a 2 year-old boy this patient was operated upon for a megacolon and when he was 13 years old for a megalocystis A subtotal thyroidectomy because of MCT was performed when he was 16 years old The following year he was operated upon for a slipped femoral epiphysis on the left side and it was noted that he had bilateral pes cavus scoliosis pectus carinatum spina bifida in S1 and Schmorl nodes in the lumbar vertebrae As it further was found that he had a marfanoid body build *prognathism dolichocephaly* and multiple mucosal neuromas the patient was classified as having MEN 2b During 1977 to 1978 serum calcitonin rose to 275 000 pmol/l PTH and 25 hydroxy calciferol in serum hydroxyproline excretion in the urine as well as the bone mineral content were all within normal ranges

Discussion

In 1968 GORLIN et coll noticed that in a few patients with MCT pheochromocytoma and mucosal neuromas marfanoid body build was an associated finding In 1975 KHAIRI et coll suggested the name MEN 3 for the combination of MCT pheochromocytoma mucosal neuromas with or without marfanoid body build the latter being defined as a slender body build with a sparsity of body fat and poor development of the body musculature occasionally accompanied by pes cavus pectus excavatum and high arched palate

CARNEY et coll (1978) surveyed their 17 patients and an additional 73 patients from the literature all having MEN 3 or as CARNEY et coll call the syndrome—MEN 2b They further included the following skeletal abnormalities prognathism elongation of the skull (dolichocephaly) and slipped femoral proximal epiphysis

In the present series only 4 patients with MCT and various bone defects were found to have mucosal neuromas i e they belong to the group of MEN 2b All 4 patients had marfanoid body build and prognathism but these were found in other patients with MCT as well

The possible causes of these bone abnormalities could be (1) a disorder in connective tissue as in patients with Marfan syndrome (2) a sustained effect of calcitonin on the bones or (3) a defect of a stem cell in the neural crest

(1) *Marfan syndrome* is an autosomal dominant inheritable disease affecting the skeletal system the aorta and the lens MCKUSICK (1972) suggested that both the defects in the aorta and the lens as well as the bone disorders could be due to a defect in connective tissue probably collagen When compared with the bone defects in patients with the genuine Marfan syndrome the following traits may occur also in patients with MCT dolichocephaly high arched palate prognathism anterior chest deformity kyphoscoliosis pes cavus spina bifida and marfanoid body build (muscular underdevelopment and sparsity of body fat) In this comparison bone cysts Schmorl nodes and slipped femoral proximal epiphysis are limited to patients with MCT

In none of the present patients could the main characteristics of the Marfan syndrome be found ectopia lentis dilation of the aorta and arachnodactyly Hydroxyproline in the urine is occasionally increased in patients with Marfan syndrome (SJOERDSMA et coll 1958) however in 5 of the patients with various bone defects including 3 patients with MEN 2b it was normal

MCT may appear as an autosomal dominant disease and in that case most often as a part of MEN 2a or to a lesser degree MEN 2b (CARNEY et coll) However most cases are non hereditary in the present series only 2 of 28 cases

(2) *Sustained effect of calcitonin* Calcitonin is a polypeptide hormone normally produced and secreted by the c-cells in the thyroid gland In cases of malignant development of the c cells i e in medullary thyroid carcinoma calcitonin is secreted in abnormal high serum concentrations The normal range of serum calcitonin is 120 to 290 pmol/l measured with the technique used in the present series In patients with MCT serum calcitonin in the range of 6000 to 275000 pmol/l was measured

The role of calcitonin in the normal human physiology is only partly known It is generally accepted that the effect is the opposite of that of parathyroid hormone with the exception of the effect on phosphate as both hormones improve the excretion of phosphate in the urine (ARDAILLOU et coll 1969 SINGER et coll 1969) SØRENSEN et coll (1970) injected porcine calcitonin into both *hypercalcemic and hypocalcemic patients and* found a lowering of serum calcium in both groups This hypocalcemic effect is probably due to an inhibition of bone resorption (BIJVOET et coll 1970)

It has not been possible to recognize any effect of depletion of calcitonin i e in patients with the entire thyroid gland removed

In hypersecretory states i e in patients with MCT a definite metabolic function of calcitonin has not been established It may have been expected that serum calcium and serum phosphate were decreased in patients with high serum calcitonin but this has not been the case as reported by MELVIN et coll (1973) and RASMUSSON (1978) The effect on bone tissue may be an increased mineral content in patients with longstanding high serum calcitonin However this was not found by MELVIN et coll who assessed the bone mineral content in 18 patients with MCT nor in 5 of the present patients who moreover had normal 25 hydrox calciferol serum calcium and PTH

In 3 patients with MCT MELVIN et coll found th all parameters related to bone cell activity we markedly decreased These findings were not co firmed in bone biopsies performed on 2 of th present patients

It is possible that the absence of a demonstrabl metabolic effect of the pathologically increase serum calcitonin in patients with MCT is due to th possibility that the encountered serum calcitonin not the metabolic active form of calcitonin but fo example is a nonactive precursor molecule o calcitonin (DONK et coll 1976) or simply anothe calcitonin reacting immunologically like the norma calcitonin in the assay used in the present series bu without the normal calcitonin effect on calcium PTH and phosphate

(3) *Stem cell in the neural crest* MCT develop from the c-cells in the thyroid gland These cell have their origin in the neural crest (LE DOUARIN & LIÈVRE 1970) and so have cells in the pituitary the pancreas the stomach the intestine and the adrena medulla The cells are named APUD-cells because they have common cytochemical properties (amin precursor uptake and decarboxylation) Tumors developed from the APUD-cells are called apudomas (PEARSE 1968) Apudomas share a common stem cell in the embryonal neural crest with neuromas schwannomas and melanomas and these tumors together are called neurolophomas after a proposal from PEARSE & POLAK (1974)

A defect in this stem cell could apart from MCT lead to multiple neuromas which may be the cause of some of the bone abnormalities occurring in patients with MCT (spina bifida and scoliosis) Further HORST (1975) has suggested that neural crest cells migrate into and become a part of the musculoskeletal blastema and hence a possible cause for the various bone abnormalities is present

SUMMARY

The frequency and appearance of bone abnormalities in patients with medullary carcinoma of the thyroid were compared with those of patients with other thyroid carcinomas Only in patients with medullary carcinoma the following abnormalities were found dolichocephaly prognathism anterior chest deformity bone cysts Schmorl nodes spina bifida slipped femoral epiphysis pes cavus and marfanoid body build Not only patients with medullary carcinoma of the thyroid as part of

multiple endocrine neoplasia but also patients with other wise unrelated medullary carcinoma had bone defects The cause of the bone abnormalities is probably a defect of a stem cell in the embryonal neural crest rather than sustained effect on bone tissue of the tumor produced calcitonin

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INVASIVE SQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX

I Definition of parameters in a histopathologic malignancy grading system

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Several attempts have been made to select treat nt modality according to histopathologic evalua n of tumour biopsies In 1893 VON HANSEMANN roduced the term anaplasia to denote an absence differentiation He believed that the degree of plasia was directly related to the invasive capaci of a tumour This principle has been followed by st authors trying to evaluate the biologic activity squamous cell carcinoma of the uterine cervix wever the designs of the classification have been st variable

SCHOTTLAENDER & KERMAUNER (1912) divided tumours into reife mittelreife und unreife (ma semi mature and immature)

BRODERS (1920 1925 1926 1940) and GOELLNER 76) classified the tumours as of high moderate d poor differentiation according to a scale from 1 4

MARTZLOFF (1923 1928) divided the tumours into nal transitional and fat spindle cell types

BÖHM & ZWEIFEL (1926) used four groups based the Schottlaender & Kermauner classification ded keratinization and suggested the value of es nating the tumour host relationship in terms of llular response eosinophilia and the amount of nnective tissue

HEALY & CUTTLER (1928) distinguished 3 grades dult plexiform and anaplastic) closely corresponding to Martzloff's grades and Broders grades 2 to 4

HUEPER (1928a b) estimated both the tumour cell population and the tumour host relationship by 20 different factors each grading from 1 to 4 the score constituting the malignancy index of tumour and correlating with survival The parameters used were (1) special cell type of carcinoma (2) nucleo-cytoplasmic coefficient (3) number of pencil cells (4) infiltrative cell growth (5) general type of carcinoma (6) irregular cell size (7) irregular cell shape (8) distinctness in outline of cells (9) chromatism of cytoplasm (10) functional activity of cells (11) irregular size of nuclei (12) irregular shape of nuclei (13) chromatism of nuclei (14) hyperchromatism of nuclei (15) number of mitoses and prophases (16) irregularity of mitoses (17) character of stroma (18) vascularity of stroma (19) type of cellular infiltration of stroma and (20) amount of cellular infiltration of stroma

WARREN (1931) and GATES & WARREN (1934) classified the carcinomas as low medium and high malignant

CHAMBERS (1935) grouped the tumours into adult spindle and transitional types with the following subgroups keratinized differentiated transitional and anaplastic The latter divided into an alveolar

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and a solid form ACKERMAN & DEL REGATO (1947) used a histologic grading from 1 to 3 (high and moderate differentiation and an anaplastic type TRUELSEN (1949) divided the squamous cell carcinomas into parakeratotic squamous cell mucosal undifferentiated and basal cell types

GLUCKSMANN & SPEAR (1945) GILMOUR et coll (1949) classified the tumours as anaplastic (A tumours) and squamous cell differentiating tumours (D tumours) In 1951 PENDL submitted his elaborate classification with 16 different subtypes

A Epidermoid carcinoma
  (1) Differentiated epidermoid carcinoma
    (I) Cutaneous type (a) with horny lamellae (b) predominantly with polygonal cells (c) predominantly with spindle shaped (basal) cells
    (II) Mucosal type (a) with all layers (*) differentiated as usual (**) with Holundermarkzellen (b) one layer predominating (*) predominantly spindle shaped (basal) cells (**) predominantly pale cells (***) cuboid columnar type of cell without gland formation (****) cuboid columnar type of cell with gland formation
  (2) Only slight differentiated epidermoid carcinoma (a) small celled intermediate type (b) large celled intermediate type
B Anepidermoid carcinoma
  (1) Transitional cell carcinoma
  (2) Undifferentiated carcinoma
C Mixed type
D True adenocarcinoma
E Special types of carcinoma

REAGAN et coll (1957) and REAGAN & WENTZ (1967) classified the tumours into large cell non keratinizing large cell keratinizing and small cell carcinoma a classification adopted by WHO (POULSEN et coll 1975) GRAHAM & GRAHAM (1962) divided the tumours according to cytologic smears before and after radiation therapy into one group with good sensitization response (good SR) and the other with poor sensitization response (poor SR) A parabasal a keratinizing a pleomorphic and a small cell type grouping was used by TWEEDALE & RODDICK (1969)

Finally SEKIMOTO et coll (1978a b) designed a classification based on combination of maturation fuctional differentiation and cell type The latter was divided into basal squamous prickle pleomorphic reserve and spindle cell type

The intention of all these proposed classification was to make it possible to predict the prognosis of the individual patient with fair certainty However the results varied and were often directly conflicting Some authors found a good correlation to survival (for review see LANGE 1960) others denied that histologic grading had any prognostic value (LANGEFIELD et coll 1964 NG & ATKIN 1973 GOELLNER JOHANSSON et coll 1976) For the individual patient it was stated that no consistent guidelines as regard the future treatment of the patients could be based on the histologic appearances alone (JOHANSSON et coll)

The light microscopic technique has been complemented with immunofluorescence and immunoperoxidase methods (BONFIGLIO & FEINBERG 1976 PERTSCHUK et coll 1977 RUBIO et coll 1978) and scanning electron microscopy (RUBIO & KRANTZ 1976 RUBIO & EINHORN 1977) Calculation of DNA content (ATKIN 1964 NØDSKOV PEDERSEN 1971) and chromosome analyses (ATKIN & BAKER 1979) have been made Computer analysis of exfoliated cells from the uterine cervix (HOLMQUIST et coll 1978) and computer aided cluster and discriminant analyses (KRAMER et coll 1974) have also been attempted These methods are still not easily accessible but the aim has been to find objective and reproducible diagnostic criteria

At the Department of Tumour Pathology of Radiumhemmet Stockholm a malignancy grading system 8 parameters each graded in 1 to 4 points was developed to evaluate the biologic activities of squamous cell carcinoma of the larynx (JAKOBSSON 1973 1975) palate (ENEROTH & MOBERGER 1973) gingiva (WILLÉN et coll 1975) This system was used by LUND et coll (1975a b 1976 1977) in carcinomas of the lip tongue and larynx and by FISHER (1975) and HELWEG LARSEN et coll (1978) for the larynx The present malignancy grading system was adopted and modified by STENDAHL et coll (1979) for squamous cell carcinoma of the uterine cervix (8 parameters each graded from 1 to 3 points one new parameter introduced) In a retrospective investigation a high correlation was found between the sum of malignancy points and the clinical outcome of the individual The present report gives a definition and a closer description of the parameters used

Table 1

Parameters used for malignancy point grading Tumour cell population

er	Points 1	2	3
Structure	Exophytic papillary and solid	Small cords and groups of cells	Marked cellular dissociation
Differentiation into cell type	Large cell no keratinization	Large cell keratinized	Small cell no keratinization
Nuclear polymorphism	>75 per cent mature nuclei few enlarged nuclei	75 to 25 per cent mature nuclei moderate number of enlarged nuclei	<75 per cent mature nuclei numerous irregular or anaplastic enlarged nuclei
Mitoses	Single 0–1	Moderate number 0–5	Numerous 0–>5

Table 2

Parameters used for malignancy point grading Tumour host relationship

r	Points 1	2	3
Mode of invasion	Well defined borderline	Cords less marked borderline	Groups of cells or diffuse growth
Stage of invasion	(Min stroma inv) or microcarcinoma	Nodular into submucosa and connective tissue	Massive amongst muscles and vessels
Vascular invasion	None	Possible	Well established within the lumina of lymph or blood vessels
Cellular response (plasmolymphocytic)	Marked (continuous rim)	Moderate (several large patches)	Slight or none (few small patches or no cells)

Methods

ie tumour cell population and the tumour–host ionship were considered separately The ıation of the tumour cell population (Table 1) based on the grading of cell differentiation cture nuclear polymorphism and the frequency itotic figures in terms of a 1 to 3 point scale The our–host relationship (Table 2) was also esti ed in terms of a 1 to 3 point scale by the mode of sion the stage of invasion vascular invasion the degree of lymphoplasmocytic infiltration se 8 morphologic parameters permitted a grad with 8 to 24 points totally

Comments

Tumour cell population

Structure (parameter 1) Magnification (4×12 5) It is important that the biopsy also includes stroma

1 point Papillary and solid types growing exo phytically Care should be taken that it is not an inverted growth with cords and groups of cells that penetrate the stroma (Fig 1)

2 points Small cords and groups of cells with re tained cohesion This group also includes aggrega tions of 15 to 20 cells sometimes combined with mesenchymal tissue (Fig 2)



Fig 1



Fig 2



Fig 3

Fig 1 Structure (parameter 1) Papillary type One point

Fig 2 Structure (parameter 1) Small cords and groups of cells with retained cohesion Two points

Fig 3 Structure (parameter 1) Marked cellular dissociation Three points



Fig 4

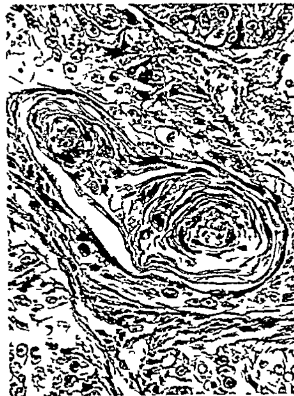


Fig 5

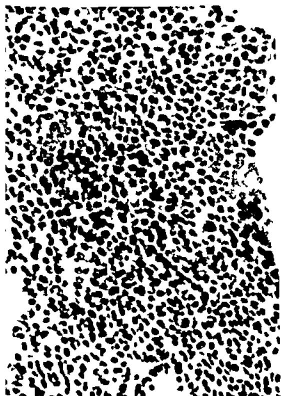


Fig 6

Fig 4 Differentiation into cell type (parameter 2) Large cell non-keratinizing type One point

Fig 5 Differentiation into cell type (parameter 2) Large cell keratinizing type with pearl formation Two points

Fig 6 Differentiation into cell type (parameter 2) Small cell non keratinizing type with marked hyperchromatism Three points



Fig 7

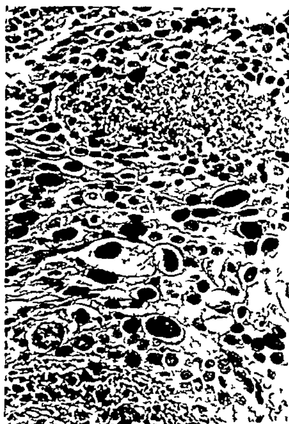


Fig 8



Fig 9

Fig 7 Nuclear polymorphism (parameter 3) Only slight nuclear variation with more than 75 per cent mature nuclei One point

Fig 8 Nuclear polymorphism (parameter 3) Less than 75 per cent mature nuclei with solitary giant tumour cells and a moderate number of large nuclei Two points

Fig 9 Nuclear polymorphism (parameter 3) Less than 25 per cent mature cells with numerous giant tumour cells also bizarre containing tumour cell forms Three points



Fig 10



Fig 11



Fig 12

Fig 10 Mode of invasion (parameter 5) Invasive squamous cell carcinoma with a defined borderline and clearly demarcated interface towards the stroma One point

Fig 11 Mode of invasion (parameter 5) Less distinct borderline with disintegrating cords but the cells are still connected to each other Two points

Fig 12 Mode of invasion (parameter 5) Strongly disintegrated appearances with individual cells or small cell groups Three points



Fig 13



Fig 14



Fig 15

Fig 13 Stage of invasion (parameter 6) Slight invasion into stroma One point

Fig 14 Stage of invasion (parameter 6) Invasive squamous cell carcinoma with growth into stroma and collagen tissue not reaching the medium sized vessels or the muscles Two points

Fig 15 Stage of invasion (parameter 6) Growth close to and between medium sized vessels and also growth into the musculature Three points



Vascular invasion (parameter 7) Invasive squamous cell ma within the vessel wall (→) but no evidence of intra manifestations Two points



7 Vascular invasion (parameter 7) Well established growth our cells within the lumen of a blood vessel Three points

3 points Marked cellular dissociation Only a few cells may cling together (Fig 3)

Differentiation into cell type (parameter 2) follows the definition given by WHO (POULSEN et coll) Generally only one cell type is found but in mixtures of several kinds for example large aggregations of basaloid cells in combination with large cell formations points are given to the lowest differentiated form (STENDAHL et coll)

1 point Large cell non keratinizing type This is the most frequent sub-type Keratinization is absent or limited to isolated cells Sometimes these tumours are composed of cells with abundant clear cytoplasm (Fig 4)

2 points Large cell keratinizing type This subtype is characterized by tumour cells with pearl formation Strongly keratinized parakeratotic forms are also included in this group even if only small amounts of keratine are found in intermediate and basal cell layers One to 3 cell layers with negligible keratinization in the surface area are classified with 1 point (Fig 5)

3 points Small cell non keratinizing type This type is rare (2 to 10 per cent) No keratinization is found The cells are smaller but the relative nuclear size is increased Hyperchromatism of the nuclei is marked (Fig 6)

Nuclear polymorphism (parameter 3) This is a modification of Broders system (BRODERS 1920 1925 1926 1940) Thirty high power fields are examined (40×12 5)

1 point Requires more than 75 per cent mature nuclei and only negligible size variations (Fig 7)

2 points Requires 25 to 75 per cent mature nuclei solitary giant tumour cells and a moderate number of enlarged nuclei (Fig 8)

3 points Requires less than 25 per cent mature cells extremely irregular often numerous giant tumour cells and bizarre tumour cell forms Extremely immature cells of basaloid type are included (Fig 9)

Mitoses (parameter 4) Magnification (40×12 5) This parameter requires very accurate classification Thirty high power fields are examined The counting must not only be related to the basal cell layer but also to the prickle-cell and superficial layers Clear chromosome pattern should be distinguishable to avoid interpreting hyperchromatic cell nuclei or pycnotic cell nuclei as mitoses

1 point 0 or 1 mitosis per high power field

2 points 2 to 5 mitoses per high power field



Fig 18 Lymphoplasmocytic cell response (parameter 8) Marked cellular reaction around and within the tumour nodules One point



Fig 19 Lymphoplasmocytic cell response (parameter 8) Unevenly distributed aggregates of lymphoplasmocytic cells in some areas nearly no lymphocytes Two points

3 points More than 5 mitoses per high power field

Tumour–host relationship

Mode of invasion (parameter 5) Magnification (10×12 5) Attention should be paid to fixation artefacts mechanical fragmentation and some disintegration because of an inflammatory reaction in connection with intensive lympho plasmocytic or lichenoid reaction

1 point Defined border line with a pushing appearance where the interface continues to be clearly demarkated When border line is slightly less distinct on account of an inflammatory reaction covering some cell layers the tumour is included in this group (Fig 10)

2 points Less distinct border line The carcinoma penetrates the stroma with pointed disintegrating cords still connected to each other (Fig 11)

3 points Strongly disintegrated appearance Individual cells or groups up to 10 cells Round aggregates containing 10 to 15 cells or more often with a marked hyaline membrane are given 1 point (Fig 12)

Stage of invasion (parameter 6) The biopsy should include all cell layers also including the lamina muscularis

1 point Minimum stromal invasion or a microcarcinoma is defined according to WHO (POULSEN et coll) Minimal stromal invasion is carcinoma in situ with areas giving suspicion of penetration into the underlying stroma Microcarcinoma is defined as stromal invasion limited to isolated microscopic foci (generally 5 mm or less in depths) (Fig 13)

2 points Growth into stroma and collagen tissue where the tumour should not penetrate deeper than one vessel diameter from vessels of medium size The border line between 2 and 3 points may sometimes be difficult to detect A deep biopsy and accurate examination of tumour growth in relation to the medium sized vessels is necessary (Fig 14)

3 points Growth close to medium sized vessels or between the vessels and growth into the musculature (Fig 15)

Vascular invasion (parameter 7) This parameter is difficult to estimate Fixation artefacts and mechanical fragmentation that easily give spaces with intima like covering must be identified



Fig 0 Lymphoplasmocytic cell response (parameter 8) Slight cell reaction in the stroma No reaction within the tumour nodules Three points

1 point No vascular invasion

2 points Growth around vessels with compressive effect or infiltrating the wall without reaching the lumen (Fig 16)

3 points Clear and well established growth of tumour cells into the lumen of lymphatics or blood vessels (Fig 17)

Lymphoplasmocytic cell response (parameter 8) Magnification (10×12 5)

1 point Marked cellular reaction with a continuous border of lymphoplasmocytic cells around the tumour nodules in many places (Fig 18)

2 points Unevenly distributed aggregates of lymphoplasmocyctic cells other areas sparsely covered with lymphocytes (Fig 19)

3 points Minimum or no lymphoplasmocytic cell reaction e g solitary small clusters minimum amount of dissociated cells or no lymphoplasmocytic cells at all (Fig 20)

Discussion

Microscopic classification of squamous cell carcinoma was presented by VON HANSEMANN SCHOTTLAENDER & KERMAUNER and BRODERS (1920 1925 1926 1940) mainly as a monofactorial system based on cellular differentiation A number of similar systems have later followed (for review see LANGE)

It was early realized that the connective tissue had a capacity to prevent progression of the tumour and the tumour–host relationship was carefully analysed SCHOTTLAENDER & KERMAUNER described different tumour–connective tissue relations medullary tumours in which the connective tissue made up a small part a scirrhous type with preponderance of connective tissue and finally mixed types Scirrhous tumours are rather uncommon and were considered to offer the best prognosis ADLER (1916) stated that apart from the type of connective tissue the vascularity of the tumour might influence the prognosis He gave 4 possible combinations of which tumours rich in connective tissue and sparsely vascularized were supposed to have the best prognoses following radiation therapy

LAHM (1927) divided the connective tissue reaction around the tumour into 4 grades Grade 1 hyperaemia and round-cell infiltration grade 2 granulation tissue in the interface between tumour parenchyma and the underlying connective tissue grade 3 no cellular reaction and grade 4 sclerotic connective tissue Prognosis was said to be best in grade 4 LAX (1950) classified the connective tissue reaction into 3 groups (1) satisfactory new formation of connective tissue with regular arrangement and ample fibres (2) poor reaction with no or little formation of fibres and (3) shrinkage hyalinization and partial breakdown of the connective tissue with rupture and splitting of the fibres In this system prognosis deteriorated from 1 to 3 LINELL & MÅNSSON (1952) evaluated the amount and type of stroma but found no relation to survival PAAVOLAINEN (1970) and PAAVOLAINEN et coll (1973) found an increased frequency of local recurrences when acid mucopolysaccharides increased in the stroma

Contrary to LAHM SCHOCK (1926) and STUPER (1953a b) LINELL & MÅNSSON could not find that a large number of eosinophilic cells was correlated to better prognosis

An increased number of granulocytes resulted in decreased survival (HUEPER & SCHMITZ 1927 HUEPER 1928a b) Tumour necrosis was also correlated to higher mortality (KOTTMEIER 1955 GLUCKSMANN & SPEAR) An enhancement of lymphocytes into the stroma was said to reduce the

frequency of metastasis (CHERRY & GLUCKSMANN 1955 GUSBERG et coll 1971)

BÖHM & ZWEIFEL LINELL & MÅNSSON as well as CULLHED (1978) reported that absence of lymphocytic infiltration was an unfavourable prognostic sign of survival However BARBER et coll (1978) found no such correlation CULLHED likewise demonstrated a significant low frequency of lymph gland metastasis when a high rate of lymphocytes around the tumour complexes was encountered The growth of the carcinoma into the underlying stroma was observed with attention paid to the mode of infiltration Tumours with ribbon and bandlike configurations had a better clinical outcome than those with complete cellular dissociation (SCHOTTLAENDER & KERMAUNER STÜPER 1953a b LAX BEECHAM et coll 1978 CULLHED)

The importance of vascular invasion is evident and patients with such a feature face worse clinical outcome in both operated and irradiated patients (STEIN WERBLOWSKY 1955 CHERRY & GLUCKSMANN BARBER et coll CULLHED)

Several multifactorial classification systems have been developed HUEPER (1928a b) devised an index of malignancy by which the sensitivity of the tumour to radiation could be assessed It was claimed to correlate well with survival The complicated classification giving points for 20 different factors made the system highly subjective and has not become widely used PENDL s classification is very elaborate but requires a large material of patients Groups 2 3 6 and 13 comprise 60 to 65 per cent of all patients (PENDL SCHULLER 1952 STUPER 1953a b) and the other 11 groups constitute the remaining 35 to 40 per cent In STÜPER s large series of 815 patients 9 of the groups comprised less than 40 patients and 4 less than 10 patients However well founded the PENDL classification may be morphologically and theoretically it does not appear to be suitable for routine use

SCHULLER demonstrated that PENDL s classification could also be used when reaching decision whether a patient should be operated upon or irradiated CULLHED used several of the parameters used in the present material but made no attempt to calculate total malignancy points The functional classification of SEKIMOTO et coll (1978a b) has been published in short communications only The clinical value remains to be proven

The present malignancy grading system includes a description of the tumour cell population and the tumour–host relationship and is an attempt to create a simple and reproducible method The parameters were chosen on the basis of the good results previously obtained by ENEROTH & MOBERGER WILLÉN et coll LUND et coll (1975a b 1976 1977) CULLHED and STENDAHL et coll but these parameters will not necessarily be those finally used The prognostic value of the parameters may also be revised A multifactorial analysis is at present in progress which will provide further information in this respect Preliminary results indicate that most of the prognostic information is found in the tumour–host relationship

It is essential that all parameters can be identified in the biopsy and that characteristics of the invasive zone are not underestimated This is in agreement with JAKOBSSON (1973) but is contrary to the results of LUND et coll (1975a b 1976 1977) and HELWEG LARSEN et coll thus implying that large and deep biopsies must be taken in order to fit into the grading system The biopsy should also be large in order to reduce the sampling error as parameters such as cell type nuclear polymorphism mitoses and mode of invasion may be unevenly distributed in the tissue (MARTZLOFF 1928 SILVERBERG 1976 CHI et coll 1977)

SUMMARY

A histologic malignancy grading system for invasive squamous cell carcinoma of the uterine cervix is presented The method is based upon evaluation of the tumour cell population in terms of cell differentiation structure nuclear polymorphism and the frequency of mitotic figures in terms of a 1 to 3 point scale The tumour host relationship was also estimated in terms of a 1 to 3 point scale by the mode of invasion stage of invasion vascular invasion and degree of lymphoplasmocytic infiltration These parameters permitted a grading with 8 to 24 points totally

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FROM THE INSTITUTO DE ONCOLOGIA Y RADIOBIOLOGIA LA HABANA CUBA

SURVIVAL IN CARCINOMA OF THE UTERINE CERVIX CORRELATED TO PRIMARY TREATMENT RESULTS

J JIMENEZ J ALERT L BELDARRAÍN
J MONTALVO and C ROCA

rcinoma of the uterine cervix is clinically gen y confined to the pelvis although distant metas to lung liver bones etc may occur Many nts die without signs of local activity Thus 'AD et coll (1979) found tumor spread into the is and abdomen in patients with an apparently ial cervix after radiation therapy

a group of patients with cervical carcinoma the ival was correlated to the effect of the irradia at the end of the radiation therapy and the result ow presented

Material and Methods

ie material consisted of 1 106 patients with car ma of the uterine cervix irradiated between 1967 1972 at this institute The method of treatment been reported previously (JIMENEZ et coll) The cases were classified according to M 186 patients (16 9%) belonged to stage I 81 %) to stage IIa 608 (54 9%) to stage IIb 176 9%) to stage III and 55 (4 9%) to stage IV

Results

he 5 year survival rate and the result at the end rradiation appear in the Table

lo clinical evidence of tumor activity was found 354 patients (77 2%) 571 (66 8%) were alive and ll 5 years after treatment

Doubtful tumor activity was considered in 135 patients (12 2%) 47 (34 8%) were alive and well 5 years after treatment

Evident tumor activity was considered to be pres ent in 117 patients (10 6%) at the end of irradiation 18 (15 4%) were alive and without signs of activity 5 years after treatment 8 of these belonged to stage III and 3 to stage IV

Discussion

At the end of the irradiation the tumor may have disappeared completely at clinical examination in other cases evident tumor tissue remains and in still another group it is doubtful whether viable tu mor tissue remained When the 5 year survival is analysed the clinical evaluation of the tumor at the end of irradiation does not give a reliable indication of the long term survival

Cytology may give an erroneous result in case of recurrence under a healthy epithelium (KRAUS & SCHUHMANN 1979) It is also possible that in a biop sy specimen small areas of viable cells may be over looked at microscopy (PILLERON et coll 1972)

In stage I the survival figures decrease when the clinical examination gives doubtful results or an evi dent tumor is found The same is valid in stages IIa and IIb

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FROM THE DEPARTMENT OF RADIATION BIOLOGY CENTRE D ÉTUDE DE L ÉNERGIE NUCLÉAIRE
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IRON INCORPORATION AFTER SINGLE AND FRACTIONATED IRRADIATION OF INFANT MICE

G B GERBER and J MAES

:ionation of a radiation dose in general per rtial repair of radiation injury and thus causes ury than the same dose delivered in a single tion However certain exceptions to this rule Thus mice exposed on day 6 and day 9 of play a higher mortality than when they are he same dose either on day 6 or on day 9 ANN & MUTH 1977) The mechanism causing isitization not yet known It may be related adaptation of hemopoiesis to adult life taking during the neonatal period (METCALF & 3 1971) a suggestion which is supported by :t that the sensitization ceases as the mice adulthood Since such an apparent lack of of radiation injury in the infant organism not . of interest on theoretic grounds but also may mplications for radiation therapy and radia otection it was considered of interest to elu this mechanism in more detail The present deals with the normal development of the opoiesis during the infancy of mice and the es caused by a single and a fractionated radia ose Incorporation of iron into hemopoietic s was utilized to assess the capacity of the opoietic system (BELCHER et coll 1954 N & TRIBUKAIT 1971 cf also review in ER & ALTMAN 1970) Later GERBER & MAES reported on the behavior of the stem cells ieir sensitivity to radiation as well as the sig nce of such a sensitization for late effects

Methods

Infant mice of the C_{57} Bl strain were whole body irradiated at 250 kV 1 mm Cu filter 0 84 Gy/min The following groups were used (1) Controls (2) exposed to 1 26 Gy on day 6 (3) exposed to 4 2 Gy on day 6 (4) exposed to 4 2 Gy on day 9 (5) exposed to 1 26 Gy on day 6 followed by 2 94 Gy on day 9 At an age of 7 8 10 14 17 20 25 or 30 days the mice were injected intraperitoneally with 37 MBq of ^{59}Fe citrate and killed 4 hours later The activity of serum and erythrocytes was determined by gamma spectrometry after separation by centrifugation in calibrated hematocrit tubes and the data were related to 0 14 ml of blood In addition the dependence on time after injection of serum and erythrocyte activities was followed for selected doses and time intervals after exposure Activities of both femurs liver and spleen and bone marrow were also assayed Practically all activity is bound to the protein fraction at this time All experimental groups were followed in at least two independent experiments 4 to 6 animals were used for each time point

Results

Preliminary experiments demonstrated that the strain used showed about the same sensitization as that of BAUMANN & MUTH All animals survived a

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dose of 1 26 Gy about 5 to 10 per cent died before an age of 30 days after a single dose of 4 2 Gy given on day 6 or 9 50 per cent of the mice given the frac tionated irradiation (1 26 Gy on day 6 2 94 Gy on day 9) had died at an age of 20 days and 80 per cent had died before an age of 30 days

The growth of infant mice is retarded by irradia tion in a dose dependent manner Whereas controls had attained a weight of 15 g at an age of 30 days mice exposed to 1 26 Gy weighed 10 g and those exposed to 4 2 Gy 7 5 g on day 30 no difference being discernible between animals irradiated with a single or a fractionated dose Similar results were observed with respect to liver or spleen weight Hematocrit values displayed a dose dependent reduction wich was more marked and longer lasting after a fractionated exposure to 4 2 Gy than after a single one (Fig 1) ^{59}Fe in serum i e that not in corporated into heme rapidly diminished with time to low levels in the controls (not shown half life time less than 30 min) Four hours after injection only traces of active iron were found in the serum of control mice whereas much higher activities re mained in that of irradiated mice maximum values being observed 1 to 3 days after exposure (Fig 2) One week after exposure serum activity had again returned to normal but in mice given a fractionated irradiation the serum iron rose again from an age of 20 days until day 30

In explaining the data on iron incorporation into heme it should be recalled that after 4 hours many cells with newly synthetized heme have already left the hemopoietic tissues and have entered the blood The time at which labelled erythrocytes appear in blood is shorter in young than in older mice and also diminishes during regeneration after irradiation Radioactivity incorporated into heme of bone marrow (Fig 3) increased in controls between day 15 and day 20 Irradiation with 1 26 Gy on day 6 caused a temporary increase in activity 1 to 3 days later whereas irradiation with 4 2 Gy on day 6 reduced incorporation in bone marrow during the following week and prevented the age related rise in non irradiated mice This is also observed following exposure to 4 2 Gy on day 9 except that the early decrease is absent Fractionated exposure caused a marked temporary increase in activity at an age of 15 days and prevented the normal increase during the third week of life

Iron incorporation into heme of control spleens (Fig 4) increased slowly up to the third week of life and then declined This rise and decline seem to be slightly delayed after 1 26 Gy However irradiation with higher doses caused an immediate fall in iron incorporation later the values approached normal

Iron incorporation into hepatic heme (Fig 5 appeared to be nearly independent of the age of the animals After irradiation this incorporation was increased to a degree which appears about inversely proportional to the fall in incorporation of iron into hemopoietic tissues

The percentage of active iron appearing in ery throcytes in 0 14 ml of blood decreased with age (Fig 6) During the initial 4 days after irradiation ^{59}Fe activity in erythrocytes diminished slightly after 1 26 Gy and markedly after 4 2 Gy the fall after a fractionated exposure was less marked than after a single one During the second week after exposure to 4 2 Gy erythrocyte activity was markedly enhanced above control levels and then returned to normal The changes after a fractionated exposure although less marked were long lasting

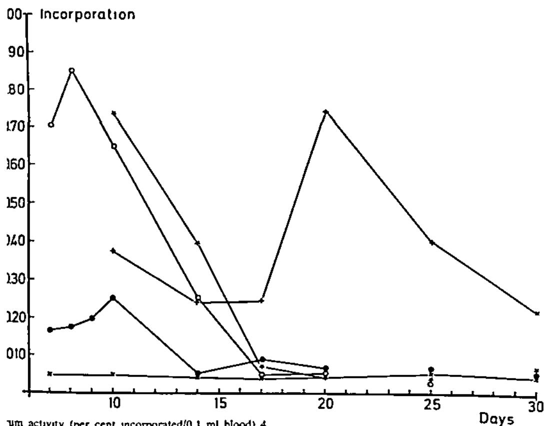
Discussion

The early period after birth is characterized by profound modifications in hemopoietic functions (METCALF & MOORE) Already before birth hemo poiesis in the liver has been replaced by that in the spleen and from the second week of life i e at a time when adult hematocrit levels are attained hemopoiesis in bone marrow takes more and more precedence to that in the spleen This is also de monstrated by the data presented Kinetic investiga tions on iron incorporation into erythrocytes (no shown in the data) indicate that the time interva until labelled erythrocytes appear after ^{59}Fe injec tion i e the maturation of heme synthetizing cells also increases with age during the neonatal period

In explaining the data after irradiation it should be kept in mind that only mice surviving at a given time are examined When severely injured mice die and are thus removed from the experimental group the mean of the surviving animals may suggest an improvement of a given parameter Moreover the relations between iron activity of erythrocytes and those in the hemopoietic organs must also be taken into account The data demonstrate that irradiation during infancy produces a bone marrow syndrome characterized by profound alterations particularly in erythropoiesis The maturation of the erythropoietic functions of bone marrow appear especially affect

40 Hematocrit
35
30
25
20
15
10
5
10 15 20 25 30
Days

ıatocrıt (ın per cent) of control and ırradıated ınfant ıe age of 7 to 30 days (× controls ● 1 26 Gy on day 6 ○ 4 2 Gy on day 6 ★ 4 ? Gy on day 9 † 1 26 Gy on day 6 fol lowed by ? 94 Gy on day 9)



ıum actıvıty (per cent ıncorporated/0 1 ml blood) 4 ınjectıon of ^{59}Fe ınto control and ırradıated ınfant ools as ın Fig 1

Fig 3 Activity (per cent incorporated in both femurs) in bone marrow of control and irradiated infant mice 4 hours after injection of ^{59}Fe Symbols as in Fig 1

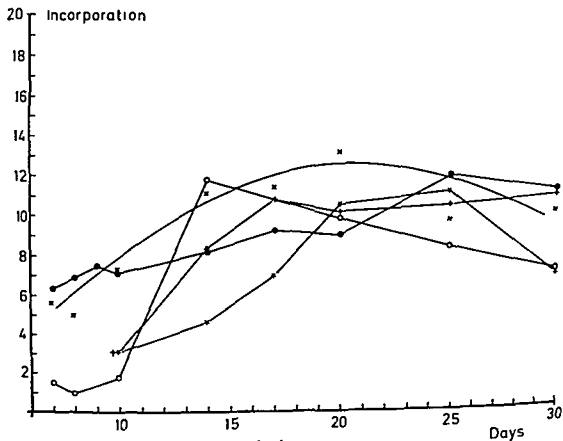
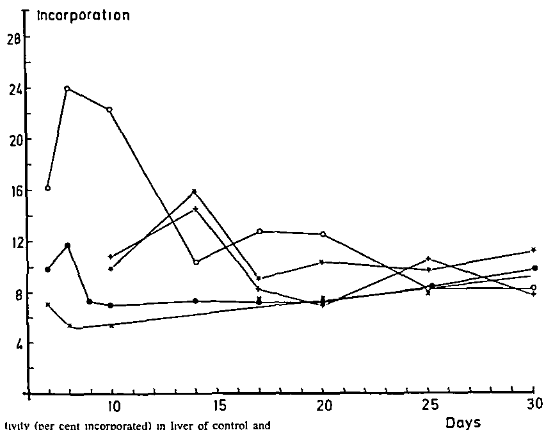


Fig 4 Activity (per cent incorporated) in spleen of control and irradiated infant mice 4 hours after injection of ^{59}Fe Symbols as in Fig 1



tivity (per cent incorporated) in liver of control and infant mice 4 hours after injection of ^{9}Fe Symbols as

Incorporation
5
4
3
2
1
10
15
20
25
30
Days

ctivity in erythrocytes (per cent incorporated) of control iated infant mice 4 hours after injection of Fe Symbols
1

ed changes in the spleen are less marked These changes are in general somewhat more marked and last longer after a fractionated than after a single exposure to the same dose An increase in iron in corporation occurring a few days after a dose of *1 26 Gy may be caused by a compensatory mech* anism

The lower turnover of serum iron during the first days after exposure reflect the lack of heme synthe tizing cells in hemopoietic organs and allow a greater deposition of iron in the liver

In conclusion the observations confirm the higher effectiveness of a fractionated irradiation during in fancy compared with a single one and indicate that these animals die from hemopoietic failure mainly due to an impaired maturation of the hemopoietic capacities of the bone marrow It remains the task of future investigations to better define the cellular basis of these alterations

SUMMARY

The erythropoiesis in control and irradiated infant mice was investigated on the basis of incorporation of iron into hemopoietic organs and blood Iron incorporation in bone marrow increased from the second week of life reflecting the maturation of the marrow whereas that in the spleen showed only minor changes Irradiation of infant mice on day 6 or 9 caused a bone marrow syndrome characterized by an impaired iron incorporation into hemopoietic tissues and blood by a reduced utilization of serum iron and particularly by a delayed maturation of the erythropoietic functions of bone marrow Fractionated irradiation not only had a greater effect on mortality but also caused a more severe and long lasting depression of erythropoiesis than a single dose

ACKNOWLEDGEMENTS

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OM THE DEPARTMENT OF PATHOLOGY THE GADE INSTITUTE UNIVERSITY OF BERGEN HAUKELAND SYKEHUS N 5016 BERGEN NORWAY

EFFECTS OF HYPERTHERMIA ON A NEUROGENIC RAT CELL LINE (BT_4C) IN CULTURE

Development of thermal tolerance during continuous heating

OLAV DAHL

Hypoxic cells are at least as sensitive to hyperthermia as well oxygenated cells (GERWECK et coll 74 SCHULMAN & HALL 1974 HARISIADIS et coll 75 POWER & HARRIS 1977 GERWECK et coll 79) This may partly be due to an increased lactic d production which lowers the pH under hypoxic nditions (OVERGAARD & SKOVGAARD POULSEN 77 OVERGAARD & BICHEL 1977) or also lack of trients (HAHN 1974 OVERGAARD 1977) The obable existence of anoxic or hypoxic subpopulations due to necrosis and hemorrhage in gliomatous nours (CHANG 1975) may contribute to the relatively poor effect of radiation therapy in these nours

The present investigation was initiated to examine e effects of supranormal temperatures (41–45 C) survival of malignant neurogenic cells in culture a basis for experimental hyperthermia in vivo

Materials and Methods

Cell line In all experiments a malignant neurogenic rat cell line designated BT_4C was used The ell line originated after a single transplacental ose (75 μg/g body weight) of N ethylnitrosourea dministered to pregnant female BD IX rats by intravenous injection on the 18th day of gestation LÆRUM & RAJEWSKY 1975) The dissociated fetal rain cells grown in long term culture became tumourigenic after about 200 days The BT_4C line is composed of bi or tripolar cells with a few giant cells and contains the specific nervous system protein S 100 When implanted subcutaneously in BD IX rats the cell line gives rise to pleomorphic or neurinoma like tumours (LÆRUM et coll 1977) The morphology was unaltered during the passages (45–70)

The BT_4C line has a doubling time of 18 to 20 hours when grown in asynchronous monolayer culture The cells have triploid DNA content when analysed by flow cytometry (LÆRUM & HANSTEEN 1975)

Cell culture The cells were grown in Dulbecco s modification of Eagle s medium (DMEM Flow Laboratories Irvine) supplemented with 10% heat inactivated newborn calf serum four fold concentration of non-essential amino acids penicillin (100 IU/ml) and streptomycin (100 μg/ml) Monolayer cultures were maintained in 5% CO in humified air at 37 C The cells were free of mycoplasma contamination when tested in agar under aerobic and anaerobic conditions

For each experiment nearly confluent 3 day old monolayer cultures were rinsed once with phosphate buffered salt solution (PBS) and trypsinized (2

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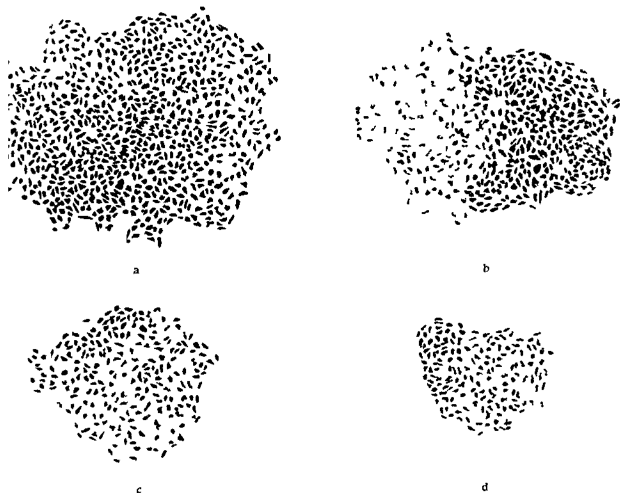
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! Photomicrographs of stained colonies 8 days after expo- o 41 0°C for different times a) Control 37 0°C b) 41 0°C for 2 h c) 41 0°C for 4 h d) 41 0°C for 12 h Decrease in size after the longer incubation times Magnification ×55 3

;s in the water bath until they were removed temperature reached a range within 0 1 C of the red temperature in 4 to 5 min when heated from ; (data not shown)

Results

he survival curves have initial shoulders fol ed by logarithmic cell killing (Fig 1) The linear t of the curves can be described by the para er D_0—the reciprocal of the slope of the curves the duration in minutes at a particular tempera : needed to reduce the survival to l/e (37%) he initial value (BHUYAN 1979) The size of the ulder is expressed by the quasi threshold dose i e the time in minutes from start of therapy ained by extrapolating the linear part of the curve surviving fraction=1 0 (HARRIS et coll 1977) e extrapolation number n is the intercept of the :ar part of the curve and the y axis The relation ween these is

$$D_q = D_0 \times \ln n$$

The values for the three curve parameters at dif ferent temperatures are given in the Table

No clonogenic cells were killed the first 8 hours at 40 0°C but after an incubation time of 12 hours a slight decrease occurred At 41 0 C the linear part of the curve was biphasic with no further cell killing between 8 and 12 hours The same biphasic re sponse was indicated at about the same survival level at 42 0 C after 2 hours This change is not caused by cell multiplication during treatment as proliferation during the hyperthermic treatment would give rise to greater colonies instead of more colonies In fact a parallel decrease in colony size occurred with longer incubation times indicating a reduced proliferative capacity of the surviving cells (Figs 2 3)

The reduction of the slope of the linear part of the curves at 41 0 and 42 0°C is probably due to

acquired resistance or thermal tolerance (defined as a decrease of the slope of the heat survival curve or an increase of D_0)

There is a great reduction of survival between 42 0 and 43 0°C This is reflected in the Arrhenius plot (Fig 4 JOHNSON et coll 1954 WESTRA & DEWEY 1971 CONNOR et coll 1977 BHUYAN) by a break of the curve at 43 0°C The inflection indicates a change in activation energy μ which can be calculated from the Arrhenius equation

$$K = 1/D_0 = A \times e^{-\mu/RT} \text{ or } K_1 = K_0 \times e^{\frac{\mu}{R}\left(\frac{T_1 - T_0}{T_1 \times T_0}\right)}$$

where K is the slope of the survival curves ($1/D_0$) and A in the present temperature range (40–45°C) is practically a constant (BHUYAN) $A = K/h \times Txe^{\Delta S/R}$ where K is Boltzmann s constant ($1\ 38 \times 10^{-16}$ erg/ K) and h is Planck s constant ($6\ 63 \times 10^{-23}$ erg/s) and ΔS the change in inactivation entropy (BAUER & HENLE 1979) R is the gas constant 8 3 kJ/mol degree (2 kcal/mol degree) and T the absolute temperature in K Inserting the values for $1/D_0$ (K_0 and K_1) at different temperatures (T_0 and T_1) in the latter equation then gives the activation energies for the cell line between 41 0 and 43 0°C the activation energy is 1 175 kJ/mol (280 kcal/mol) and between 43 0 and 45 0 C the activation energy is 356 kJ/mol (85 kcal/mol) for heat killing of BT_4C cells

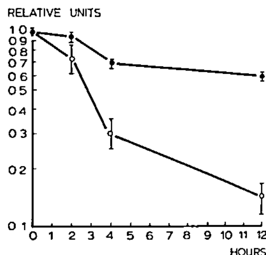


Fig 3 Relative colony size as function of the incubation time at 41 0°C Each point is the mean of 40 measured colonies after 8 days incubation at 37°C ●=relative diameter of colonies (mean of controls 1 0) O=relative cell number (calculated by multiplying surviving fraction and colony area) Mean ± SEM

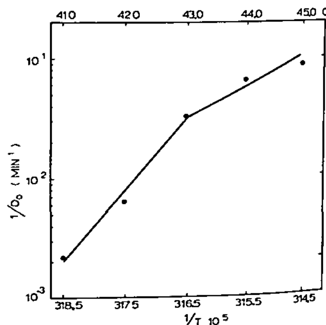


Fig 4 Arrhenius plot for heat inactivation of BT C cells The reciprocal values for D are plotted versus the reciprocal of the absolute temperature (°K)

Discussion

The survival of the neurogenic rat cell line BT_4C after exposure to elevated temperatures in culture is dependent on the temperature as well as the duration of the treatment as stated over 50 years ago both in vitro (LAMBERT 1912) and in animal experiments (WESTERMARK 1927)

Shoulders exist in the heat survival curves (Fig 1) as found for most cell lines (BHUYAN) The contribution to the shoulders by the temperature equilibrium time (5 min) is negligible below 41 0°C but with the decrease of the shoulders at the higher temperatures the contribution of the equilibrium time also increases Complex mechanisms like inherent cell resistance to heat or possible ability to accumulate and repair heat damage are also involved

The heat survival curve parameters D_0 as an expression of the initial linear part of the curves presented in the Table are in accordance with the results for several cell lines examined in vitro (ROBINSON & WIZENBERG 1974 BHUYAN) The BT_4C cells are more sensitive to heat than two human astrocytoma lines (GERWECK & BURLETT 1978) examined in vitro

Colony size Decrease in colony size as a function of the duration of hyperthermia (Figs 2 3) is re

acquired resistance or thermal tolerance (defined as a decrease of the slope of the heat survival curve or an increase of D_0)

There is a great reduction of survival between 42 0 and 43 0 C This is reflected in the Arrhenius plot (Fig 4 JOHNSON et coll 1954 WESTRA & DEWEY 1971 CONNOR et coll 1977 BHUYAN) by a break of the curve at 43 0°C The inflection indicates a change in activation energy μ which can be calculated from the Arrhenius equation

$$K = 1/D_0 = A \times e^{-\mu/RT} \text{ or } K_1 = K_0 \times e^{\frac{\mu}{R}\left(\frac{T_1 - T_0}{T_1 \times T_0}\right)}$$

where K is the slope of the survival curves ($1/D_0$) and A in the present temperature range (40–45°C) is practically a constant (BHUYAN) $A = K/h \times T x e^{\Delta S/R}$ where K is Boltzmann s constant (1 38× 10^{-16} erg/ K) and h is Planck s constant (6 63× 10^{-3} erg/s) and ΔS the change in inactivation entropy (BAUER & HENLE 1979) R is the gas constant 8 3 kJ/mol degree (2 kcal/mol degree) and T the absolute temperature in K Inserting the values for $1/D_0$ (K_0 and K_1) at different temperatures (T_0 and T_1) in the latter equation then gives the activation energies for the cell line between 41 0 and 43 0 C the activation energy is 1 175 kJ/mol (280 kcal/mol) and between 43 0 and 45 0 C the activation energy is 356 kJ/mol (85 kcal/mol) for heat killing of BT_4C cells

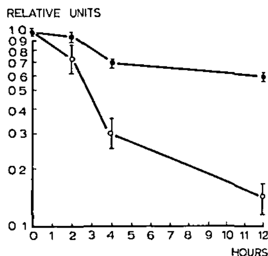


Fig 3 Relative colony size as function of the incubation tir 41 0°C Each point is the mean of 40 measured colonies af days incubation at 37°C ●=relative diameter of colonies (r of controls 1 0) O=relative cell number (calculated by mult ing surviving fraction and colony area) Mean ± SEM

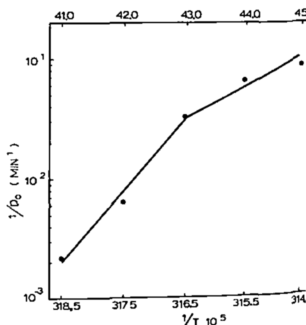


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A mouse cell line (NIELSEN & OVERGAARD) which does not develop thermal tolerance during continuous heating clearly shows thermal tolerance by fractionated heating Whether these two types of thermal tolerance are caused by the same mechanism is not known

In vivo Thermal induced temporary resistance or tolerance to a second heat treatment in vivo was originally reported for mouse tumours (S91 melanoma and C_5 Bl/6 sarcoma CRILE JR 1961 1963) as well as normal tissue (loss of foot) when preheated at 44 C one day before the second treatment

By using split dose hyperthermia (SUIT 1977) on normal tissue (loss of foot in mice) and on a fibrosarcoma minimum repair was found the first 5 hours after exposure to 43 5 C but the resistance increased at 22 hours interval When 10 fractions instead of one were used separated by 22 hours 5 3 and 4 4 times longer total exposure times were necessary to give the same result on normal tissue and tumours respectively

The effect of two separate heat treatments on normal tissue (necrosis of heated mouse ears) has recently been investigated (LAW et coll 1979a b) She had to increase the total heating time at 43 5 C in order to maintain the same level of injury if the interval between the fractions exceeded 4 hours The additional time increased until at 20 to 24 hours the second treatment was equal to the single dose time indicating full repair of the injury caused by the first treatment At longer intervals the second exposure had to be still longer reaching a maximum at 48 hours then a decay started with full extinction of the effect of the first dose after 96 hours She also found that the time of maximum thermal tolerance was dependent on the heat dose (time at 43 5 C)

Fractionated heating also induces resistance to thermal enhancement of radiation injury in vivo (LAW et coll 1978 1979a)

Underlying mechanisms Thermal tolerance as defined is a concept describing the thermal induced change in slope of the heat survival curve i e the heat induced resistance to heating The term does not reveal the underlying mechanism although the conditions which promote or inhibit its development as well as the kinetics involved give some information Based on experiments several generalisations can be made The development of thermal tolerance takes time (2–20 hours GERWECK & ROTTINGER 1976 HARISIADIS et coll 1977 SAPARETO et coll GERNER et coll 1979 LAW et coll 1979b) reaching a maximum at 8 to 48 hours (HENLE et coll 1978 1979 LAW et coll 1979b NIELSEN & OVERGAARD) then fading off in 1 to 2 days (HENLE & LEEPER LAW et coll 1979b NIELSEN & OVERGAARD) Thermal tolerance is not induced at 0°C (GERNER & SCHNEIDER) and it is inhibited by lowering pH (GERWECK NIELSEN & OVERGAARD) as well as inhibiting the protein synthesis by cycloheximide (LEEPER et coll 1977) These observations are some of the rationale of the hypothesis of an underlying metabolic process (GERNER & SCHNEIDER GERNER et coll 1976 HENLE et coll 1978 LEEPER et coll) possibly involving proteins or enzymes The cell membrane may also be involved in development of thermal tolerance (REEVES HENLE & LEEPER LI & HAHN 1978)

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INDICES

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